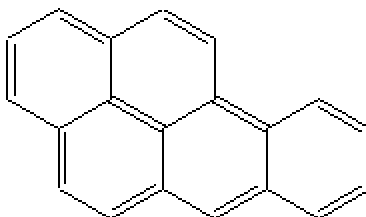


## Polycyclic Organic Matter

(including, but not limited to: benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, dibenz[*a,j*]acridine, dibenz[*a,h*]acridine, 7H-dibenzo[*c,g*]carbazole, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, fluoranthene, 2-methyl fluoranthene, 3-methyl fluoranthene, indeno[1,2,3-*cd*]pyrene, 5-methylchrysene, naphthalene, 1-nitropyrene, 4-nitropyrene, 1,6-dinitropyrene, 1,8-dinitropyrene, 6-nitrochrysene, 2-nitrofluorene, chrysene, dibenz[*a,h*]anthracene, 7,12-dimethylbenzanthracene, 3-methylcholanthrene, 5-nitroacenaphthene)



Benzo[*a*]pyrene: 50-32-8

### I. Physical and Chemical Properties - Benzo[*a*]pyrene

<i>Description</i>	Yellow crystalline solid: volatile at elevated temperatures.
<i>Molecular formula</i>	C <sub>20</sub> H <sub>12</sub>
<i>Molecular weight</i>	252.3
<i>Air concentration conversion</i>	1 ppm = 10.3 mg/m <sup>3</sup>

The compound benzo[*a*]pyrene is one of the most extensively studied members of the class of Polycyclic Aromatic Hydrocarbons (PAHs). This class, along with various PAH derivatives important as environmental pollutants, is also described as Polycyclic Organic Matter (POM). POM has been identified as a Toxic Air Contaminant by the Air Resources Board. POM consists of over 100 identified compounds, and is defined by the Federal Clean Air Act as organic compounds with more than one benzene ring that have a boiling point greater than or equal to 100°C. The usual definition of a PAH specifies that the compounds are hydrocarbons with no hetero-atom substituents or ring members, and that the compound include at least two (or, according to some authors, three) concatenated aromatic (usually benzene-like) rings. Although benzo[*a*]pyrene is one of the more abundant members of the class, it comprises no more than 5 percent of the total PAHs present in the atmosphere (Ronis *et al.*, 1983). PAHs, and derivatives such as nitro-PAHs and PAH quinones, are included under the TAC category of POM. Additionally, although naphthalene is included within the Federal Clean Air act definition of POM, it is also separately listed as a Federal hazardous air pollutant and thus as a California TAC. In this summary, the toxicity of PAHs and nitro-PAHs is reviewed for

potential impacts on the health of children and infants, with specific reference to those PAHs identified or evaluated as carcinogens by U.S. EPA, IARC, and the California TAC program (see Section V.C.) Additional specific compounds, and mixtures containing PAHs, were considered where data indicate carcinogenic or other biological activities similar to those seen for the identified carcinogenic PAHs, especially where these data include evidence of differential impacts on infants and children.

Most of the POM occurring as air pollution is attached to particulate matter. Compounds with two rings (e.g., naphthalene), although solid at room temperature in bulk, are sufficiently volatile that as air pollutants they occur in the vapor phase. Compounds with three to four rings (e.g., pyrene) occur either in the vapor phase or bound to particles, depending on the temperature and pressure. Compounds with five rings (e.g., dibenzo[*a,h*]anthracene, benzo[*a*]pyrene) exist as particles in the atmosphere (Atkinson, 1995). PAHs may also exist as solids in soil or sediment (ATSDR, 1993). PAH-derivatives include nitro-PAHs, amino-PAHs, oxygenated PAHs (phenols, quinones), and heterocyclic aromatic compounds containing nitrogen, sulfur and oxygen (Finlayson-Pitts and Pitts, 1986).

## II. Overview

There are a number of toxicological endpoints associated with PAHs to which infants and children may be especially susceptible.

- There is a general concern, based on mechanistic arguments and experimental and epidemiological data for a number of carcinogens, that exposure to a carcinogen early in life may have a greater overall impact than a similar exposure to an adult. Numerous investigators have used neonatal exposures in rodents, which have generally been found to show greater sensitivity to carcinogenesis (i.e. the number of compounds showing a statistically significant effect, the incidence and latency of tumors, and the sites affected), relative to adults (Vesselinovitch *et al.*, 1979). In addition, specific data are available showing increased sensitivity to PAHs and derivatives in young animals. A number of PAHs, PAH derivatives and mixtures containing PAHs have been identified as carcinogens in animals or humans.
- Comparative studies of the relative susceptibility to carcinogenesis at different ages have been reported. Intraperitoneal injections of benzo[*a*]pyrene to infant (day 1 or day 15) or young adult (day 42) mice produced greater lifetime incidence of lung and liver tumors in the mice treated at younger ages (Vesselinovitch *et al.*, 1975). When 3 doses of fluoranthene, 2- or 3-methylfluoranthene were injected into newborn CD-1 mice, there were high incidences of liver and lung tumors following a short latency period (Lavoie *et al.*, 1994). When 8 doses of 1-nitropyrene, 1,3-dinitropyrene, 1,6-dinitropyrene or 1,8-dinitropyrene were injected into newborn CD rats, there were increases in various tumors, some of which occurred after a very short latency period (Imaida *et al.* 1995). In addition, 1,6-dinitropyrene or 1,8-dinitropyrene produced an early increase in leukemia. These experiments are described in detail in a later section of this summary.

- Several studies in animals and humans indicate that prenatal exposure to PAHs results in serious or irreversible effects in the fetus, including cancer, teratogenesis and low birthweight. As discussed in the introductory section of this report, fetal damage sustained as a result of exposure to environmental toxicants is a source of adverse postnatal health impacts, and therefore falls within the scope of this report.
- Transplacental carcinogenesis by PAHs is a well-known phenomenon (Sram *et al.*, 1998; Anderson *et al.*, 1995). In one experiment, this was associated with induction of a specific mutation in the Ha-*ras* proto-oncogene in the fetus, which can be expressed post-natally with appropriate promotion (Yamasaki *et al.*, 1987). Thus, it appears that the mechanisms underlying transplacental carcinogenesis are similar to those for carcinogenesis after postnatal exposure, but the sites of tumor appearance are frequently more diverse, and the sensitivity is greater (Nikonova, 1977).
- Several non-carcinogenic effects have been observed following exposure *in utero* to PAHs or to mixtures containing them. These included teratogenesis (Shum *et al.*, 1979; Feuston and Mackerer, 1996), low birth weight in humans (Perera *et al.*, 1998; Dejmek *et al.*, 2000) and rodents (McKee *et al.*, 1987b), immunotoxicity (Urso *et al.*, 1992), loss of fertility in rodents exposed to benzo[a] pyrene *in utero* (Mackenzie and Angevine, 1981), human transplacental exposure resulting in hemolytic anemia (Zinkharn and Childs, 1958; Anziulewicz *et al.*, 1959) and disruption of lymphocyte maturation and hematopoiesis (Holladay and Smith, 1994). These occurred at doses at which maternal toxicity (other than long-term effects such as carcinogenesis) is minimal or absent. In several cases the effects observed after exposure *in utero* parallel toxic effects in the adult (e.g. immunotoxicity, reproductive toxicity, myelotoxicity), but whereas the effects are reversible after exposure of the adult, exposure of the fetus results in an irreversible effect.
- In addition to differential sensitivity to toxic effects in young animals and humans, there is greater exposure of children to environmental PAHs (Chuang *et al.*, 1999). Children's daily doses of PAHs (per kg of body weight) were generally higher from all routes of exposure than those of adults in the same household (Chuang *et al.*, 1999) or city (Ptashekas *et al.*, 1996). PAH-DNA adducts have been detected in the human placenta (Everson *et al.*, 1986, Weston *et al.*, 1989), the umbilical cord blood of newborns (Whyatt *et al.*, 1989), and the fetuses of experimental animals (Withey *et al.*, 1992; 1993).
- Based on these observations, studies in humans and animals suggest that children may be more sensitive to the toxic effects of PAHs and PAH derivatives (including, but not limited to, benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, dibenz[a,j]acridine, dibenz[a,h]acridine, 7H-dibenzo[c,g]carbazole, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, fluoranthene, 2-methyl fluoranthene, 3-methyl fluoranthene, indeno[1,2,3-cd]pyrene, 5-methylchrysene, naphthalene, 1-nitropyrene, 4-nitropyrene, 1,6-dinitropyrene, 1,8-dinitropyrene, 6-nitrochrysene, 2-nitrofluorene, chrysene, dibenz[a,h]anthracene,

7,12-dimethylbenzanthracene, 3-methylcholanthrene, 5-nitroacenaphthene), and mixtures containing PAHs.

### III. Principal Sources of Exposure

PAHs and other POM components are produced by the incomplete combustion of any carbon-containing materials, including fossil fuels and vegetable matter. PAHs have been detected in exhaust from both gasoline and diesel powered motor vehicles, smoke from residential wood combustion, and fly ash from coal-fired electric generating plants (Finlayson-Pitts and Pitts, 1986). Burning of vegetable materials and other waste is responsible for 50 percent of the total statewide emissions of benzo[*a*]pyrene. Other sources of benzo[*a*]pyrene, such as residential wood combustion, coal combustion, and residual oil combustion, are responsible for about 15 percent of the total statewide emissions. Some sources of PAHs and other POM components are non-anthropogenic; these can be formed during any naturally occurring combustion, such as forest fires (U.S. EPA, 1994).

Benzo[*a*]pyrene and other PAHs occur in crude oils, shale oils, and coal tars, and are emitted with gases and fly ash from active volcanoes (HSDB, 1995). In spite of these natural sources, the most important contributors to air pollution by PAHs are usually anthropogenic.

#### A. *Mobile sources*

In California, mobile sources contribute more than 35 percent of the total benzo[*a*]pyrene emissions. (Benzo[*a*]pyrene in air is quantifiable by standardized methods and is frequently used as a marker for emissions of a range of PAHs and other POM components.) Within the mobile source category, light duty vehicles are responsible for 30 percent of benzo[*a*]pyrene emissions, while heavy duty vehicles contribute approximately 10 percent (OEHHA, 1993). Before the introduction of catalytic converters (around 1974), mobile sources were the major contributor of benzo[*a*]pyrene emissions. The decreasing number of older, more polluting vehicles, and the introduction of low and even zero emission vehicles and clean fuels as part of California's Motor Vehicle Program has led to a significant and continuing reduction of POM emissions (including benzo[*a*]pyrene and other PAHs) from light-duty vehicles. Emissions from on-road mobile source diesel exhaust PM<sub>10</sub> in California are expected to decline by approximately 50 percent from 1990 until about 2010 as a result of mobile source standards and regulations adopted by the ARB through 1996 (ARB, 1998). However, the proportional reductions in emissions per mile from heavy-duty (primarily diesel powered) vehicles have so far been less dramatic than those from gasoline-powered vehicles. This has focused attention on the role of diesel exhaust as an important source of air pollutants, including both volatile and particulate POM, especially in metropolitan areas (SCAQMD, 2000). Stationary sources using diesel engines are also significant sources in some areas. Additional efforts to address this issue include the replacement of diesel powered heavy and medium-duty vehicles with clean-fuel alternatives (such as those using methanol or compressed natural gas), and the use of particle traps and other pollution reduction technologies on new and existing diesel engines. However, the impact of these changes has so far been limited.

## **B. Stationary Sources**

The primary stationary sources that have reported emissions of benzo[*a*]pyrene or other PAHs in California are paper mills, manufacturers of miscellaneous wood products, industrial machinery manufacturers, petroleum refining and the wholesale trade in petroleum and petroleum products (ARB, 1997). Estimated total emissions of PAHs from stationary sources in California are about 370,000 pounds per year, based on data reported under the Air Toxics "Hot Spots" Program (AB 2588). Table 1 lists the emissions of some individual PAHs. In addition there are approximately 2,600 pounds of unspecified POM and 250,000 pounds of unspecified PAHs reported as emissions from Hot Spots facilities. (ARB, 1997).

**Table 1: California Emissions for Individual PAHs**

<b>Compound</b>	<b>Emissions (pounds/year)</b>
Acenaphthene	6
Acenaphthylene	27
Anthracene	285
Benzo[ <i>a</i> ]pyrene*	304
Benz[ <i>a</i> ]anthracene*	175
Benzo[ <i>b</i> ]fluoranthene*	175
Benzo[ <i>k</i> ]fluoranthene*	181
Benzo[ <i>g,h,i</i> ]perylene	10
Chrysene*	275
Dibenz[ <i>a,h</i> ]anthracene*	211
Dibenz[ <i>a,e</i> ]pyrene*	3
Fluoranthene	23
Fluorene	32
Indeno[1,2,3- <i>cd</i> ]pyrene*	204
Naphthalene	360,000
Phenanthrene	63
Pyrene	42

(Data from ARB, 1997)

\* Chemicals for which PEFs are available (OEHHA, 1993)

OEHHA reviews risk assessments submitted under the Air Toxics "Hot Spots" Program (AB 2588). Of the risk assessments reviewed as of April 1996, PAHs were a major contributor to the overall cancer risk in 43 of the approximately 550 risk assessments reporting a total cancer risk equal to or greater than 1 in 1 million, and contributed to the total cancer risk in 166 of these risk assessments. PAHs also were the major contributor to overall cancer risk in 8 of the approximately 130 risk assessments reporting a total cancer risk equal to or greater than 10 in 1 million, and contributed to the total cancer risk in 54 of these risk assessments (OEHHA, 1996).

### **C. Ambient Concentrations**

California Air Resources Board's air toxics network monitors several PAHs routinely. Table 2 gives the network's mean concentration of various PAHs from January 1996 through December 1996 (ARB, 1998). The population-weighted annual ambient concentration of benzo[a]pyrene in California was estimated as 0.53 ng/m<sup>3</sup> based on 1988 to 1989 monitoring data (ARB, 1997). There are no Air Resources Board ambient measurements of naphthalene. However, Atkinson (1995) measured 12 hour average ambient concentrations of naphthalene in Redlands, California in October 1994. The levels observed ranged from 348 to 715 ng/m<sup>3</sup>. The overall mean concentration for POM from several study areas throughout the United States during 1984-91 was 8.4 ng/m<sup>3</sup> (U.S. EPA, 1993).

**Table 2: California Ambient Concentrations of PAHs**

PAH Compound	Mean Concentration (ng/m <sup>3</sup> )
Benzo[a]pyrene	0.194
Benzo[b]fluoranthene	0.245
Benzo[g,h,i]perylene	0.619
Benzo[k]fluoranthene	0.100
Dibenz[a,h]anthracene	0.031
Indeno[1,2,3-cd]pyrene	0.327

*(Data from ARB, 1997)*

### **D. Indoor Air**

Benzo[a]pyrene and other POM components are significant as indoor air pollutants. According to two large field studies conducted in California, the major sources of indoor PAHs are tobacco smoking, wood burning in fireplaces and wood stoves, and infiltration of polluted outdoor air (ARB, 1992; Sheldon *et al.*, 1993). The largest field study was conducted in northern California, in which 13 PAHs were measured inside 280 homes during the winter. Concurrent outdoor samples were collected at each home for 24 hours. The homes were selected based upon the occupants' use of tobacco, fireplaces, wood stoves, and gas heat. Table 3 lists the average indoor concentrations for some PAHs for each type of combustion source.

Average indoor PAH levels ranged from about one-fourth to 6 times the average of outdoor levels. When compared to concentrations inside homes with no obvious combustion sources ("no source"), substantially higher concentrations of all 13 PAHs were measured inside homes where smoking occurred. In addition, wood burning in fireplaces and wood stoves appeared to cause slight to moderate increases in indoor concentrations of benzo[a]anthracene, chrysene, benzo[a]fluoranthene, and benzo[a]pyrene. Investigators estimated that infiltration of polluted outdoor air was also a major contributor to indoor concentrations of PAHs, particularly outdoor air polluted by wood smoke (Sheldon *et al.*, 1993).

**Table 3: Average PAH Concentrations in Northern California Homes (ng/m<sup>3</sup>)**

PAH Compound(s)	Smoking	Fireplace	Woodstove	Gas Heat	No Source
Benzo[a]pyrene	2.2	1.0	1.2	0.41	0.83
Benzo[e]pyrene	1.1	0.49	0.55	0.25	0.42
Indeno[1,2,3-cd]pyrene	2.8	1.7	1.9	0.92	1.4
Benzo[ghi]perylene	2.0	1.4	1.5	0.78	1.3
Pyrene	4.1	2.0	2.5	1.6	1.8
Chrysene	2.0	0.56	0.61	0.24	0.4
Fluoranthene	4.5	1.9	2.3	1.4	1.6
Benzo[a]anthracene	1.3	0.43	0.55	0.17	0.32
Benzofluoranthenes	3.7	1.6	2.0	0.81	1.5

*(Data from Sheldon et al., 1993)*

Another field study measured 12 PAH compounds inside 125 southern California homes during a relatively warm fall season. At each home, two consecutive 12-hour samples were collected. Concurrent samples were also collected outside 65 of those homes. Average indoor PAH concentrations ranged from about one-half to two times the corresponding outdoor levels. Table 4 shows average concentrations (combined daytime/nighttime) for some PAHs. Levels of most PAHs were significantly higher in homes where smoking occurred than in nonsmokers' homes. As in the northern California study, investigators estimated that infiltration of polluted outdoor air was a major source of PAHs indoors (ARB, 1992).

**Table 4: Average PAH Concentrations in Southern California Homes (ng/m<sup>3</sup>)**

PAH Compound(s)	Indoor Average	Outdoor Average
Benzo[a]pyrene	0.70	0.30
Benzo[e]pyrene	0.39	0.28
Indeno[ghi]perylene	1.1	0.51
Benzo[ghi]perylene	2.4	1.0
Pyrene	2.8	2.2
Chrysene	0.30	0.39
Fluoranthene	2.2	2.5
Benzo[a]anthracene	0.16	0.18

*(Data from ARB, 1992)*

### ***E. Exposures to Children***

There is evidence that children are more heavily exposed to PAHs than adults (on a body-weight adjusted basis), and thus may suffer disproportionately from their impact, whether or not they are more susceptible to PAH toxicity. This is a result of the body size and activity patterns of children, higher breathing rates (on a body weight adjusted basis, especially for infants), and their propensity for greater dust and soil contact than adults. In particular, children in low-income families may have high exposures to PAHs. Such exposures could result from household proximity to heavy traffic or industrial sources, environmental tobacco smoke, contaminated house dust or soil, among others.

A series of studies (Chuang *et al.*, 1999) in Durham, NC and adjacent rural areas were conducted to estimate total PAH exposure of children in low-income families, and the relative importance of the environmental pathways for PAH exposure (Table 5).

**Table 5: Potential daily dose of carcinogenic (US EPA B2) PAHs  
(ng/kg b.w./day)**

Pathway	Average	S.D.	Minimum	Maximum
<i>Adults</i>				
Inhalation	1.77	3.29	0.12	15.3
Non-diet ingestion	1.28	0.91	0.38	4.24
Diet	16.3	16.7	1.61	60.9
Total	19.4	16.8	4.12	62.0
<i>Children</i>				
Inhalation	3.93	4.88	0.37	19.6
Non-diet ingestion	8.88	6.21	2.62	29.2
Diet	24.8	23.7	1.35	97.0
Total	37.6	23.7	12.2	107

(Data from Chuang *et al.*, 1999)

Higher indoor PAH levels were observed in the smokers' homes compared to nonsmokers' homes. Higher outdoor PAH levels were found in the inner city versus rural areas. Airborne PAHs deposit on soil and household surfaces, thus contributing to PAH exposures from dust (via ingestion, and inhalation of re-suspended dust) and food. The relative concentration trend for PAH in dust and soil was: house dust > entryway dust > pathway soil. The PAH concentrations were generally higher in adults' than in children's food samples. Children's potential daily doses of PAH were higher than those of adults in the same household, when intakes were normalized to body weights. Inhalation is an important pathway for children's exposure to total PAH because of the high levels of naphthalene present in both indoor and outdoor air. Ingestion pathways became more important for children's exposure to the subset of PAHs ranked as B2 (probable human carcinogens) by the U.S. EPA (see Section V.C.), most of which are of low volatility. These PAHs are included in our proposed listing. The analysis of variance results showed that inner city participants had higher total exposure to B2 PAHs than did rural participants.

Such differences between children and adults may be even more noticeable in highly contaminated environments. A monitoring program (Ptashekas *et al.*, 1996) which included benzo[*a*]pyrene was carried out in two Lithuanian cities, Vilnius, the capital of the country, and Siauliai during 1991 to 1995. Higher amounts of benzo[*a*]pyrene were found in the urine of children compared to adults, in both the control and high-risk zones. Both this and the previous study indicate that environmental PAH exposures of children result in higher body burdens than for adults in the same environments.

Crawford *et al.* (1994) examined biomarkers of environmental tobacco smoke in preschool children and their mothers. There were increases in the biologically effective dose of the carcinogenic (PAH) components of ETS in children exposed to ETS, as assessed by levels of PAH-albumin adducts. Tang *et al.* (1999) confirmed these findings in further studies of molecular and genetic damage from environmental tobacco smoke in young children, and found not only increases in protein adducts with PAHs and 4-aminobiphenyl, but also of sister-chromatid exchanges in peripheral lymphocytes of Hispanic and African-American children with home exposure to environmental tobacco smoke. Thus children exposed to ETS show increases not only in general tobacco-related biomarkers such as cotinine, but in biomarkers specific for PAHs and in genetic damage.

Airborne PAHs may contribute to exposure of children by non-inhalation routes. For example, breast milk is a route of exposure in infancy. Few studies of PAH occurrence in breast milk have been carried out, but Somogyi and Beck (1993) described a study conducted in the Federal Republic of Germany that found a number of single PAH compounds at concentrations of 5-15 ng/kg milk and, among these, benzo[*a*]pyrene, was detected at a concentration of 6.5 ng/kg. This is consistent with the findings of West and Horton (1976), who observed transfer of polycyclic hydrocarbons from the diet to milk in rats, rabbits, and sheep. Interestingly, this was more extensive in the rat (an omnivore like humans) than in the herbivorous rabbit and sheep. Lavoie *et al.* (1987) also observed transfer of benzo[*a*]pyrene and two tobacco-specific carcinogens into the milk of lactating rats.

#### ***F. Exposures in utero***

Exposure of the fetus to airborne PAHs can occur via transplacental transfer from the mother. This contributes to the body burden of PAH and PAH-DNA adducts in the child. A study (Klopov, 1998) of pregnant women in the Russian Arctic region found substantial maternal exposure to PAHs, and detected PAHs in most samples of cord blood and placenta analyzed. The results support the barrier role of the placenta, because levels of many PAH compounds in cord blood were lower than in maternal blood. Nevertheless, these compounds were passing through the placental barrier, at least partially, with the concentrations of some PAHs (anthracene and benzo[*e*]pyrene) higher in cord blood than in maternal blood. Autrup *et al.* (1995) and Autrup and Vestergaard (1996) measured the polycyclic aromatic hydrocarbon-albumin adduct level in serum isolated from the mother and the umbilical cord. The median maternal/fetal adduct ratio was approximately 1.3 (maternal blood/umbilical cord blood) and a positive association between the adduct levels in the mother and umbilical cord blood was observed.

These observations are consistent with the observation of PAH compounds and their metabolites in the fetus after maternal exposure in experimental animal studies (Withey *et al.*, 1993, Withey *et al.*, 1992; Kelman and Springer, 1982; Kihlstrom, 1986). Howard *et al.* (1995) showed that 1-nitropyrene was transported both across the placenta and into milk in mice following oral or intraperitoneal dosing. 0.7% of the administered dose crossed the placenta as 1-nitropyrene and/or its metabolites, and accumulated in the fetuses and amniotic fluid, with both C-oxidized and nitro-reduced metabolites being detected.

Everson *et al.* (1986) reported detection of smoking-related covalent DNA adducts in human placenta. Weston *et al.* (1989) isolated PAH-DNA adducts specifically identified as r-7,t-8,t-9,c-10-tetrahydroxy-7,8,9,10-tetrahydroBaP residues from human placenta. Arnould *et al.* (1997) also reported detection of benzo[a]pyrene-DNA adducts in human placenta and umbilical cord blood. Determination of tobacco consumption by urinary cotinine established that among smokers, adducts were found in 13 placenta specimens (from 10 to 60 fmol/50 µg DNA) and 12 umbilical cord blood samples (from 10 to 22.15 fmol/50 µg DNA). Thus a mother's tobacco consumption is linked to the accumulation of benzo[a]pyrene-DNA adducts in the placenta and cord blood. These adducts are seen in smaller quantities in the umbilical cord blood, probably because of the metabolic capacity of the placenta which impacts transfer of benzo[a]pyrene from the mother to the fetus.

Zenzes *et al.* (1999) investigated whether benzo[a]pyrene diol epoxide-DNA adducts are detectable in pre-implantation embryos, and studied their relationship to parental smoking. Seventeen couples were classified by their smoking habits: (i) both smokers; (ii) wife non-smoker, husband smoker; and (iii) both non-smokers. Their 27 embryos were exposed to a monoclonal antibody that recognizes benzo[a]pyrene diol epoxide-DNA adducts. The proportion of stained blastomeres was higher for embryos of smokers than for non-smokers (0.723 versus 0.310). The mean intensity score was also higher for embryos of smokers ( $1.40 \pm 0.28$ ) than for non-smokers ( $0.38 \pm 0.14$ ;  $P = 0.015$ ), but was similar for both types of smoking couples. The mean intensity score was positively correlated with the number of cigarettes smoked by fathers ( $P = 0.02$ ). Increased mean immunostaining in embryos from smokers, relative to non-smokers, indicated a relationship with parental smoking. The similar levels of immunostaining in embryos from both types of smoking couples suggest that transmission of modified DNA is mainly through spermatozoa. Paternal transmission of modified DNA was confirmed by detection of benzo[a]pyrene diol epoxide-DNA adducts in spermatozoa of a smoker father and his embryo.

The results of Whyatt *et al.* (1998) indicate that PAH-induced DNA damage in mothers and newborns is increased by ambient air pollution. These investigators measured PAH-DNA adducts in maternal and umbilical white blood cells of 70 mothers and newborns from Krakow, Poland. The modulation of DNA adduct levels by genotypes previously linked to risk of lung cancer was also investigated. There was a dose-related increase in maternal and newborn adduct levels with ambient air pollution at the women's place of residence among subjects who were not employed away from home ( $p=0.05$ ). Maternal smoking (active and passive) significantly increased maternal ( $p<0.01$ ), but not newborn adduct levels. Neither the CYP1A1 MspI nor the GSTM1 polymorphism was associated with maternal adducts. However, adducts were significantly higher in newborns heterozygous or homozygous for the

CYP1A1 MspI RFLP compared to newborns without the RFLP ( $p=0.04$ ). In the fetus, DNA damage appears to be enhanced by the CYP1A1 MspI polymorphism. A novel feature of this study was the measurement of PAH-DNA adduct levels in white blood cells of mother-newborn pairs. Transplacental exposures to PAHs are generally an order of magnitude lower than maternal exposure. The finding that levels of adducts in newborns were similar to those in mothers (in spite of the protective effects of metabolism by maternal and placental enzymes) suggests an enhanced susceptibility to DNA damage in a fetus compared to the mother.

In a separate analysis, Whyatt *et al.* (1998) determined PAH-DNA adduct levels in mother-new born pairs from Limanowa, a rural area outside Krakow where ambient pollution levels are lower but where home use of coal for heating is significantly greater. Among the 67 pairs analyzed, mean adduct levels in the newborns significantly exceeded those in the mothers. This suggests that adduct formation and the resulting DNA damage caused by maternal exposure to PAHs could be amplified in the fetus.

#### **IV. Potential for Differential Effects**

##### **A. Summary of Key Human Studies**

###### *a) Carcinogenicity*

Systematic studies addressing the relative sensitivity of infants and children to carcinogenesis by PAHs were not identified in the scientific literature. However, there is an enormous literature on the carcinogenicity of polycyclic hydrocarbons, and various mixtures containing them that may be encountered in the workplace or the general environment. Indeed, the observations of Pott (1775) on scrotal cancer in chimney sweeps (who were mostly children, and were exposed to soot from coal fires, a rich source of PAHs and other POM) are generally regarded as the first objective account of chemical carcinogenesis in humans.

###### *b) Developmental Toxicity*

Developmental toxicity has been identified as a process having impacts on children's health, as noted in the introductory chapter of this report. PAH effects reported in animals have included obvious anatomical abnormalities (see Section IV.B.b.1), but perhaps because of the less extreme exposures of humans in polluted environments compared to the animal toxicity experiments, these have not been clearly associated with human PAH exposures. However, more subtle changes such as morphometric abnormalities indicative of developmental delay, and intrauterine growth retardation (IUGR) leading to low birth weight infants have been reported both for populations living in polluted environments, and for mothers who smoke or are exposed to secondhand tobacco smoke. Recent studies of these effects are described below. While these exposures involve complex mixtures with many toxic components, the detailed associations shown, the finding of chemical-specific DNA adducts in affected offspring (see Section V.A) and the concordance with animal effects implicate PAHs as important causative toxicants for these effects. Low birth weight and developmental delay are associated with adverse experience of morbidity and mortality in childhood (and also with adverse health impacts later in life), so it is of

particular concern that well-documented reports of IUGR in humans exposed to PAH-polluted air have appeared.

Perera *et al.* (1998) studied developmental effects of fetal exposure to PAHs via ambient pollution. The study was carried out in an industrialized area of Poland with relatively high levels of PAH pollution from coal burning. PAH-DNA adducts in leukocytes and plasma cotinine were measured in umbilical cord blood, as dosimeters of transplacental PAH and cigarette smoke, respectively. The study subjects were 70 newborns from the industrialized city of Krakow and 90 newborns from Limanowa, a rural town with far greater use of coal for home heating. Newborns whose levels of PAH-DNA adducts were above the median ( $3.85/10^8$  nucleotides) had a significantly decreased birth weight, birth length, and head circumference (Table 6). Cotinine was also significantly inversely associated with birth weight and length.

**Table 6: Birth outcomes in Polish Newborns.**

	Birth Weight (g)		Birth Length (cm)		Head Circumference (cm)	
Group	Difference	P value	Difference	P value	Difference	P value
Krakow	- 205	0.11	-1.8	0.02 *	-0.9	0.05 *
Limanowa	-129	0.16	-0.8	0.17	-1.2	0.0004 *
All	-147	0.05 *	-1.1	0.02 *	-0.9	0.0005 *

(Data from Perera *et al.*, 1998)

Difference between those with high (above median) and low (below median) leukocyte levels of PAH-DNA adducts.

Dejmek *et al.* (2000) found that exposure to carcinogenic PAHs in air pollution during early pregnancy was associated with an increased adjusted odds ratio for low birth weight ("intrauterine growth retardation" - IUGR). Birth outcomes were studied over a four-year period in two towns in Bohemia (Czech Republic): Teplice (1100 births/yr) and Prachatice (450 births/year). Teplice is located in an industrialized area with surface mining of brown coal, chemical industry and large coal-fired power plants; in this area the level of general air pollution, including both particulate material (PM) and PAHs, is high. Prachatice on the other hand is located in a more rural and mountainous area without major industrial activity, and the general level of pollution (including PM) is much lower in this area. However, there is a single large point source of PAH emissions in the town. At both locations, levels of air pollution by PM and PAHs varied seasonally and over the duration of the study. Air pollution levels were measured continuously at both locations. Seven specific PAHs identified by IARC as potentially carcinogenic to humans (c-PAHs: chrysene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, indeno[1,2,3-c,d]pyrene) were measured. These PAHs are identified as carcinogenic in our TAC identification document (OEHHA, 1993).

IUGR was defined as birth weight below the 10<sup>th</sup> percentile, by sex and gestational week, in the general Czech population. Of 3,349 pregnancies at Teplice, 322 (9.6%) exhibited IUGR. At Prachatice, 124

(8.2%) of 1505 pregnancies were affected. (The study cohorts were limited to full-term pregnancies of mothers of European origin). When births were categorized according to the exposures to PM<sub>10</sub>, PM<sub>2.5</sub> and PAH, there was a significant association with exposure to air pollution, specifically to the carcinogenic PAHs. In Teplice the PAH and PM levels are highly correlated so the effects of these two pollutants could not be distinguished at this site alone. However, at Prachatice there is much less particulate pollution relative to the amount of PAH, and by comparing results at both sites it was concluded that the effect was associated with the PAH content of the pollution, not the PM<sub>(10 or 2.5)</sub>. Results relating incidence of IUGR to pollution experienced in the first gestational month are presented in Table 7 as adjusted odds ratios (AOR) for the medium and high exposure categories, relative to the low exposure category. These adjusted odds ratios were calculated using a logistic regression model in which adjustments were applied for the identified confounding variables: parity, maternal age and height, pre-pregnancy weight, education, marital status, month-specific maternal smoking, season, seasonal and long-term conception rate effects, and year of study.

The association between exposure to PAH and incidence of IUGR was only significant when exposure during the first month of gestation was considered (AOR for medium exposure = 1.63, 95% CI 0.87 - 3.06,  $p < 0.13$ ; AOR for high exposure = 2.39, 95% CI 1.01 - 5.65,  $p < 0.045$ ). Exposure at other times had no consistent effect, although a possible weak association with exposure during the eighth month was noted in Teplice only. This was interpreted as indicating that the induction of IUGR by PAH exposure resulted from an early developmental effect: this is consistent with other data suggesting that IUGR results from specific impairments of placental development soon after implantation (Zhang et al., 1995). Trends of IUGR vs. PAH exposure during the first month of exposure were highly significant in Teplice; each 10 ng increase in PAH exposure level resulted in an increase in AOR of 1.22 (95% CI 1.07 - 1.39,  $p < 0.004$ ). A similar trend was observed in Prachatice, although it did not achieve statistical significance by this analysis.

The presence of other materials besides POM in air pollution and cigarette smoke makes it difficult to definitively state the impact of PAHs on birth weight and development. However, there was specific correlation of these outcomes with PAH-DNA adducts. There is extensive evidence from other studies showing PAH-DNA adducts in humans exposed to air pollution or cigarette smoke; and there are animal studies where developmental delay and low birth weight are seen after exposure to pure PAHs. This evidence makes a causal relationship between low birth weight and developmental changes and exposure to PAHs highly plausible.

### *c) Acute Toxicity in Children*

Hemolysis has been reported in infants exposed to very high doses of naphthalene (Siegel and Wason, 1986). The effect appears to be caused by the metabolites (1- and 2- naphthol and naphthoquinones), which produce methemoglobinemia. This mode of action implies a potential for differential sensitivity of infants, due to their reduced capacity for methemoglobin reduction compared to adults. However, even in infants the doses required to produce this effect are much greater than could plausibly result from airborne levels of naphthalene; the case reports generally involved absorption by the dermal or oral routes. Zinkharn and Childs (1958) reported examining an infant exhibiting acute hemolytic anemia that

was only exposed during gestation. His mother had inhaled and ingested mothballs containing naphthalene during pregnancy (especially during the last trimester). However, it was not possible to provide an estimated infant exposure.

**Table 7: Adjusted Odds Ratios of Intrauterine Growth Retardation by c-PAHs and PM<sub>10</sub> in the first gestational month in Teplice and Prachatice.**

Pollutant	Specification	District	Medium <sup>a</sup>		High <sup>a</sup>	
			AOR <sup>b</sup>	95% CI	AOR <sup>b</sup>	95% CI
PM <sub>10</sub>	—	Teplice	1.44	(1.03–2.02)	2.14	(1.42–3.23)
	—	Prachatice	2.11	(1.03–4.33)	1.09	(0.49–2.46)
c-PAHs	—	Teplice	1.59	(1.06–2.39)	2.15	(1.27–3.63)
	—	Prachatice	1.49	(0.81–2.73)	1.26	(0.60–2.63)
	<7.5 km (n=551) <sup>c</sup>	Prachatice	1.89	(0.56–6.29)	2.44	(0.60–9.83)
	Lower cut-offs <sup>d</sup>	Prachatice	1.63	(0.87–3.06)	2.39	(1.01–5.65)

*Data from Dejmek et al. (2000)*

- Cutoffs for PM<sub>10</sub>: Low = < 40 µg/m<sup>3</sup>, Medium = 40 to < 50 µg/m<sup>3</sup>, and High = ≥50 µg/m<sup>3</sup>.  
Cutoffs for Carcinogenic PAHs (c-PAHs): Low = < 15 ng/m<sup>3</sup>, Medium = 15 to < 30 ng/m<sup>3</sup>, and High = ≥30 ng/m<sup>3</sup>.
- Medium to Low and High to Low, adjusted for parity, maternal age and height, pre-pregnancy weight, education, marital status, month-specific maternal smoking, season, seasonal and long-term conception rate effects, and year of the study.
- Only mothers living up to 7 km from the monitor station in Prachatice region.
- c-PAHs: Low = < 2 ng/m<sup>3</sup>; Medium = 2 to < 20 ng/m<sup>3</sup>; High = ≥20 ng/m<sup>3</sup>.

## **B. Summary of Key Animal Studies**

### *a) Carcinogenicity*

The carcinogenicity of PAHs and other POM constituents in animals is well known; the literature, which is very extensive, has been evaluated by IARC (1987, 1989). It is generally considered both on theoretical grounds and as a result of experimental evidence that exposures to carcinogens early in life may result in higher tumor yields. Shorter average times to tumor, and a wider range of sensitive sites, have also been reported following early in life exposures in some experiments. As discussed in the introduction to this report, there is concern that children may suffer adverse health consequences including enhanced rates of cancer from exposure to any carcinogen, due to the increased sensitivity *in utero* and early in life. There is particular concern where specific evidence of enhanced sensitivity at younger ages exists for the specific compound or class of compounds considered.

Although in the standard rodent bioassay protocol exposure begins at the young adult stage, neonatal or young rodents have often been used in carcinogenesis bioassays, including the early carcinogenesis

studies by Innes *et al.* (1969). It has generally been supposed that starting exposure at an early age would maximize the sensitivity of the assay, and this has been observed in practice (Vesselinovitch *et al.*, 1979). In some cases, such as the widely used neonatal Strain A mouse lung adenoma assay (Stoner *et al.*, 1984), the combination of neonatal exposure, a highly sensitive strain and selective endpoint definition has been used to produce a result in a matter of weeks. This contrasts with the two years usually required for a bioassay using the standard NTP protocol.

Vesselinovitch *et al.* (1975) studied the carcinogenicity of benzo[a]pyrene in two hybrid strains of mice exposed by a single intraperitoneal injection at 1, 15 or 42 days old. Tumors were observed at several sites, and the relationship between tumor incidence and age at exposure varied from site to site. In the case of liver and lung tumors, young mice were more sensitive than older mice, showing both higher tumor incidence and shorter time before appearance of tumors. Incidence of tumors was generally higher for liver and lung tumors than for other sites.

The strains used were C57BL/6J x C3HeB/FeJ F<sub>1</sub> ("B6C3F<sub>1</sub>") and C3HeB/FeJ x A/J F<sub>1</sub> ("C3AF<sub>1</sub>"); treated group sizes varied from 30 to 62 animals whereas control groups contained 98 to 100 animals. Doses of 75 or 150 µg benzo[a]pyrene were dissolved in trioctanoin. Control animals had low mortality; controls included two groups, in one of which survivors were necropsied at age 90 weeks and in the other survivors were necropsied at age 142 weeks. Incidences of all tumors in controls were low, except for lung tumors in the C3AF<sub>1</sub> mice (Males: 49/97 at 90 weeks; 60/100 at 142 weeks. Females: 26/100 at 90 weeks; 50/100 at 142 weeks). Treated animals were examined regularly for tumors throughout life; mortality immediately after dosing was virtually zero, but later survival was impacted by the appearance of lethal tumors.

Results are presented in Table 8. For liver tumors in both strains, the incidence was greater, and the average age of tumor appearance was lower, in animals treated at 1 day than at 15 days. This trend continued when comparing those treated at 42 days. The authors judged these differences to be significant ( $P < 0.01$ ) using the  $\chi^2$  test for incidence comparisons and Student's t-test for comparing averages at which tumors were detected at autopsy. Similar trends were observed for lung tumors, although the high background incidence of this tumor in the C3AF<sub>1</sub> mice reduced the extent and statistical significance of the differences.

Lavoie *et al.* (1994) examined the tumorigenic activity of fluoranthene, 2-methylfluoranthene and 3-methylfluoranthene in newborn CD-1 mice. All three compounds were assayed at intraperitoneal doses of 3.46 and 17.3 µmol, given during the course of three injections on days 1, 8 and 15 after birth. Effective group sizes were between 16 and 34 (alive at 1 year: survival was about 50%). The bioassay was terminated when mice were 1 year old. Fluoranthene, a compound of interest as an air pollutant, is inactive as an initiator in the skin-painting assay (with phorbol ester promotion) in the adult mouse, which is often regarded as a particularly sensitive assay for the carcinogenic activity of PAHs. Among the five isomers of methylfluoranthene, only 2-methylfluoranthene (2-MeFA) and 3-methylfluoranthene (3-MeFA) are active as tumor initiators on adult mouse skin. But fluoranthene and 2-MeFA induced lung tumors in both male and female neonatal mice (Table 9).

**Table 8: Incidence of lung and liver tumors in mice treated with benzo[*a*]pyrene at various ages**

Dose:		Liver tumors				Lung tumors					
		75mg/kg		150 mg/kg		75mg/kg			150 mg/kg		
Strain/ Sex	Age when dosed <sup>a</sup> (days)	% tumors <sup>b</sup>	Time to tumor <sup>c</sup> (weeks)	% tumors <sup>b</sup>	Time to tumor <sup>c</sup> (weeks)	% tumors <sup>b</sup>	Time to tumor <sup>c</sup> (weeks)	Multi- plicity <sup>d</sup>	% tumors <sup>b</sup>	Time to tumor <sup>c</sup> (weeks)	Multi- plicity <sup>d</sup>
<b><i>B6C3F<sub>1</sub></i></b>											
Males	1	55	86	81	81	43	103	3	59	84	4
	15	60	93	58	81	25	103	2	36	82	2
	42	13	108	9	87	36	119	2	38	95	2
Females	1	7	129	18	121	49	126	3	62	112	4
	15	7	116	7	90	33	122	2	40	101	3
	42	0		0		26	131	2	17	118	3
<b><i>C3A<sub>F1</sub></i></b>											
Males	1	34	80	46	69	93	78	6	92	70	8
	15	27	90	23	77	93	87	5	94	75	6
	42	0		3	79	93	91	5	87	85	6
Females	1	2	91	2	70	93	82	7	93	73	7
	15	2	102	2	62	94	98	5	91	79	6
	42	0		0		87	93	5	90	83	6

(Data from Vesselinovitch et al., 1975)

- Age (in days) at which animals received i.p. injections of BP at the stated dose level, dissolved in trioctanoin.
- Number of mice bearing liver or lung tumors / effective number exposed, expressed as a percentage.
- Average age (in weeks) at which tumors were observed.
- Average number of grossly visible lung tumors per whole lung.

**Table 9: Carcinogenicity of fluoranthenes in neonatal mice.**

Compound	Dose	Newborn Mouse			Adult mouse results (skin bioassays)
		Sex	Lung Tumors %	Liver Tumors %	
Fluoranthene	17.3 $\mu$ mol	F	86***	7	-ve
		M	65**	100***	
	3.46 $\mu$ mol	F	35*	0	
		M	43*	64***	
2-Methyl Fluoranthene	17.3 $\mu$ mol	F	69***	31***	+ve
		M	96***	92***	
	3.46 $\mu$ mol	F	18	3	
		M	16	45*	
3- Methyl Fluoranthene	17.3 $\mu$ mol	F	21	11*	+ve
		M	19	69***	
	3.46 $\mu$ mol	F	15	0	
		M	25	33	
DMSO Control	17.3 $\mu$ mol	F	12	6	
		M	17	17	

(Data from LaVoie et al., 1994)

P: \*\*\* < 0.001, \*\* < 0.005, \* < 0.05

Fluoranthene, 2-MeFA and 3-MeFA when administered to newborn mice also induced a significant incidence of liver tumors among male mice, although only 2-MeFA was tumorigenic in the liver of female mice.

The findings in this study are typical of those obtained in neonatal rodent experiments, in that tumors appeared after a relatively short latency, at multiple sites. As in the case of other similar bioassays using the neonatal mouse model, it is notable that a high yield (up to 100% for some compound, site and sex combinations in the high-dose groups of this experiment) is obtained after only three injections early in life, with a very small total dose. While the testing of these compounds in adults is inadequate, the limited data available suggest that the carcinogenic response in adults is not so strong. In adult bioassays, dosing even with potent carcinogens must usually be continuous or repeated for several months, or else continued doses of promoters applied (as in the case of the skin studies reported for these fluoranthenes), before a statistically significant incidence of tumors is observed. LaVoie et al. (1994) also commented, in comparing the results of this study with those of earlier similar experiments, that although a substantial tumor yield was obtained with studies which were terminated earlier, this experiment showed increased appearance of tumors due to the continuation of the study into mid-life (1 year old). They also noted an apparent increase in the grade of the lesions (carcinomas vs. adenomas) in the longer experiment, which supports the concept that the lesions observed in the adult mice after

neonatal exposure are the result of progression of lesions created, but not necessarily evident, in infancy, soon after the exposure.

Another bioassay, reported by Imaida et al. (1995), illustrates a typical experimental design where the bioassay is started with neonatal rodents to maximize the sensitivity and shorten the duration of the experiment. This was performed with 1-nitropyrene, and 1,3-, 1,6- and 1,8-dinitropyrene; except for 1,3-dinitropyrene, these PAH derivatives have established cancer potency equivalency factors (OEHHA 1993; OEHHA, 1999). (In other experiments reported in the same paper, the effects of 4-nitropyrene, and phenolic metabolites of 1-nitropyrene were also described.) Newborn female CD rats received subcutaneous injections of 1-nitropyrene or dinitropyrenes at 8 weekly intervals starting on the day of birth (total dose = 6.3  $\mu$ mol). Controls received injections of the solvent, dimethyl sulfoxide, only. The rats were killed at 67 weeks. As shown in Table 10, the dinitropyrenes induced malignant fibrous histiocytoma (MFH) at the site of injection. 1,6- and 1,8-dinitropyrene produced 100% incidences of this tumor, and also induced leukemia with incidences of around 20%. 1-nitropyrene induced a 33% incidence of mammary tumors.

**Table 10: Induction by 1-NP and dinitropyrenes of MFH, mammary tumors and leukemia in newborn female CD rats by s.c. injection**

Compound <sup>a</sup>	Effective no. of rats	No. of rats with MFH (%)	Average MFH induction period (d)	No. of rats with mammary tumors (%)	Average mammary tumor induction period (d)	No. of rats with leukemia (%)	Average survival period (d)
1-NP	49	0	-	16 (33) <sup>b</sup>	441	0	481
1,3-DNP	43	5 (12) <sup>b</sup>	247	9 (21)	472	0	468
1,6-DNP	46	46 (100) <sup>d</sup>	115	5 (11)	150	9 (20) <sup>c</sup>	149
1,8-DNP	37	37 (100) <sup>d</sup>	122	5 (14)	143	8 (22) <sup>c</sup>	164
DMSO	40	0	-	8 (20)	450	0	495 <sup>e</sup>

(Data from Imaida et al., 1995)

- a The animals received eight s.c. injections with nitropyrenes, at weekly intervals starting on the day of birth (total dose = 6.3  $\mu$ mol). The rats were killed at 67 weeks.
- b  $p < 0.05$  as compared to the solvent control.
- c  $p < 0.005$ .
- d  $p < 0.0001$ .
- e One animal had a carcinoma of the Zymbal gland.

MFH = malignant fibrous histiocytoma NP = nitropyrenes DNP = dinitropyrene DMSO = dimethyl sulfoxide.

In all cases the average time to tumor was short compared to a standard two-year bioassay, in which tumors are usually observed mostly towards the end of the experiment. This was particularly notable for 1,6- and 1,8-dinitropyrene, where the average time to tumor for both the MFH and the leukemia was between 100 and 150 days. (The leukemias were either fatal or, if incidental, observed at necropsy, so

the average survival time is taken as time to tumor in this case.) Wislocki et al. (1986) obtained similar results with regard to induction of lung tumors in neonatal mice for a number of nitroaromatics, including nitropyrenes. The total doses used were somewhat lower, but smaller incidences of tumors were observed. Also, the neonatal mouse lung adenoma bioassay is generally considered to be an even more sensitive test system than the neonatal rats used by Imaida et al. (1995). The experiments by Wislocki et al. (1986) were used by OEHHHA (1993) in calculating potency equivalence factors for some nitropyrenes.

Since these PAH derivatives induce leukemia, a cancer to which children are known to be particularly susceptible, there may be further cause for anticipating a differential impact of these pollutants on children, although it is not known how, or if, these agents may interact with other causes of childhood leukemia. The possible differential impact of leukemogenic agents on infants and children is discussed in the introductory section of this report.

*b) Developmental Toxicity*

*(1) Teratogenesis*

As noted in the discussion of developmental toxicity in the introduction to this report, the induction of either anatomical or functional terata is considered an effect having an adverse effect on the health of infants and children. Similarly, where such effects are noted in animal experiments, this is supporting evidence of the potential for impacts on the health of infants and children. Postnatal mortality is clearly an effect within these terms of reference, but fetal mortality (usually identified as resorptions in animal experiments) is not. However, the occurrence of pre-natal mortality should be noted as an indicator of fetotoxicity, since many fetotoxins (including PAHs, as described below) produce a spectrum of effects. These include anatomical and functional teratogenesis, prenatal mortality, perinatal mortality (stillbirth), postnatal mortality, growth retardation and developmental delay. The combination of these outcomes observed in a particular experiment may depend on dose level and timing, test species used, and other experimental conditions.

Intraperitoneal benzo[a]pyrene, at doses between 50 and 300 mg 1 mg/kg body weight given at day 7 or 10 of gestation, causes *in utero* toxicity and teratogenicity in mice (Shum *et al.*, 1979). A reduction in the number of surviving offspring (resulting both from resorptions and stillbirths) was observed in all cases. The severity of the effect was correlated with the ability of the fetus and maternal systems to metabolize benzo[a]pyrene, which is influenced by induction of aryl hydrocarbon hydroxylase (AHH). Mice (and other mammals) are described as genetically "responsive" when AHH activity is induced by exposure to PAHs and other activators of the *Ah* receptor. A greater impact on pre- and post-natal mortality was observed in C57BL/6 mice which are responsive to AHH induction than in non-responsive AKR inbred mice. Malformations were noted in the responsive mice only; these included club foot, hemangioendothelioma, cleft palate and various other anomalies of the skeleton and soft tissues. Representative results from this study are shown in Table 11.

**Table 11: Impact of benzo[a]pyrene treatment on fetal and newborn survival and malformations in responsive and non-responsive mice.**

Dosed on Gestation Day	Strain	# Litters	# Implant-ations	# Stillborn	# Resorptions	# Malformed	% all effects
7	B6	7	48	2	19	17	79
	AK	6	43	0	9	0	21
10	B6	10	62	0	11	18	47
	AK	11	78	1	8	0	12
12	B6	7	47	1	20	3	51
	AK	5	45	0	6	0	13
Control	B6	31	187	2	33	0	19
	AK	12	107	0	6	0	6.5

(Data from Shum *et al.*, 1979)

B6 = C57BL/6 mice, responsive to AHH induction.

AK = AKR mice, non-responsive to AHH induction.

Dose given was 200 mg/kg benzo[a]pyrene i.p

With the use of AKR x (C57BL/6) (AKR)F<sub>1</sub> and (C57BL/6) (AKR)F<sub>1</sub> x AKR backcrosses, it was shown that allelic differences at the *Ah* locus in the fetus could be correlated with dysmorphogenesis. If the mother is non-responsive (*Ah<sup>d</sup>/Ah<sup>d</sup>*), the *Ah<sup>b</sup>/Ah<sup>d</sup>* genotype in the fetus is associated with more stillborns, and resorptions, decreased fetal weight, increased congenital anomalies, and enhanced P<sub>1</sub>-450-mediated covalent binding of BP metabolites to fetal protein and DNA, when compared with the *Ah<sup>d</sup>/Ah<sup>d</sup>* genotype in the fetus from the same uterus. If the mother is responsive (*Ah<sup>b</sup>/Ah<sup>d</sup>*), however, none of these parameters can be distinguished between *Ah<sup>b</sup>/Ah<sup>d</sup>* and *Ah<sup>d</sup>/Ah<sup>d</sup>* individuals in the same uterus, presumably because enhanced BP metabolism in maternal tissues and placenta cancels out these differences between individual fetuses.

Other investigators have also shown teratogenesis as a result of exposure *in utero* to PAHs, including mixed materials such as are often encountered as environmental contaminants. Feuston and Mackerer (1996) administered clarified slurry oil (CSO), a refinery stream produced by processing crude oil, to pregnant Sprague-Dawley rats on gestation days [GD] 9-12, via dermal application, at doses of 0, 10, 100, and 1000 mg/kg. Maternal toxicity was evident in the dams exposed to CSO, but clear evidence of developmental toxicity was observed at 1000 mg/kg. The effects seen included increased embryolethality, decreased body weight, and anomalous development (cleft palate, brachydactyly, edema). A low incidence of abnormal fetal development was observed at 100 mg/kg. Three to seven-ring polycyclic aromatic compounds are present in CSO, and the authors considered that these PAHs were responsible for the developmental toxicity.

McKee *et al.* (1987a;b) reported reproductive and subchronic toxicity studies of liquids derived from liquefaction of coal. These materials were of interest as a potential novel route to liquid fuels, but the

products contain a number of polycyclic aromatic hydrocarbons, which are concentrated in the high-boiling fractions of the coal-derived liquids. Following treatment of pregnant Sprague-Dawley rats with 0.02, 0.1, 0.5 or 1 g/kg of coal-derived fuel oil or recycle solvent, significant fetal growth retardation was observed, as well as the induction of a small number of diverse skeletal and visceral terata. Results of an experiment with "EDS recycle solvent", a PAH-containing fraction from the coal liquefaction process, are shown in Table 12; significant reductions in number, crown-rump length and weight of fetuses were observed at 0.5 and 1.0 g/kg.

**Table 12: Fetal measurements following exposure *in utero* to EDS recycle solvent**

Measurement <sup>a</sup>	Dosage group			
	Control	Low (0.1 g/kg)	Mid (0.5 g/kg)	High (1.0 g/kg)
Number of male fetuses / litter	5.96 ± 2.05 (49)	5.79 ± 1.69 (24)	3.96* ± 2.37 (25)	0.13** ± 0.46 (23)
Number of female fetuses / litter	5.61 ± 2.06 (49)	5.67 ± 1.52 (24)	3.64* ± 2.23 (25)	0.35** ± 0.57 (23)
Crown-rump length: male fetuses (mm)	3.60 ± 0.16 (292)	3.58 ± 0.15 (139)	3.37*** ± 0.17 (99)	3.06*** ± 0.28 (3)
Crown-rump length: female fetuses (mm)	3.52 ± 0.18 (275)	3.48 ± 0.19 (136)	3.27*** ± 0.20 (91)	3.07*** ± 0.19 (8)
Weight of male fetuses (g)	4.23 ± 0.32 (288) <sup>b</sup>	4.22 ± 0.30 (139)	3.82*** ± 0.41 (99)	2.79*** ± 0.45 (3)
Weight of female fetuses (g)	4.07 ± 0.33 (273)	3.96 ± 0.38 (136)	3.47*** ± 0.38 (91)	3.07*** ± 0.35 (8)

(Data from McKee *et al.*, 1987)

a Results expressed as the group mean ± S.D. (n)

b There were 6 fetuses in 1 litter which were measured, but not weighed.

\* p < 0.05 by ANOVA.

\*\* p < 0.01 by ANOVA.

\*\*\* Significantly different from controls (P < 0.05) by standard nested analysis of variance.

## (2) Developmental reproductive toxicity

Infertility was observed in CD-1 mice after exposure *in utero* to benzo[a]pyrene (Mackenzie and Angevine, 1981). Groups of 30 or 60 pregnant female mice were given doses of 10, 40 or 160 mg/kg/day benzo[a]pyrene in 0.2 ml corn oil on days 7 through 16 of gestation; controls received corn oil only. There was no maternal toxicity or embryoletality at any dose level, although pregnancy maintenance was impaired at 160 mg/kg/day. Mean pup weight was reduced in the litters of all treated dams, with the effect becoming more noticeable with age. As adults, offspring which were exposed to benzo[a]pyrene *in utero* showed loss of fertility in controlled breeding studies with untreated partners:

at the higher doses this included complete infertility, and histological abnormalities of the gonads. Treated pup weights, and results of the breeding studies with the F<sub>1</sub> mice are shown in Table 13.

**Table 13: Pup weight and reproductive performance of male and female F<sub>1</sub> mice exposed prenatally to benzo[a]pyrene**

	Benzo[a]pyrene (mg/kg/day) <sup>a</sup>			
	0	10	40	160
<b>Treated Pup Weight</b>				
Mean pup weight at 4 days (g)	2.7 ± 0.02	2.8 ± 0.04	2.5 ± 0.02	2.2 ± 0.04
Mean pup weight at 20 days (g)	11.2 ± 0.1	11.6 ± 0.1	10.4 ± 0.1**	9.7 ± 0.2**
Mean pup weight at 42 days (g)	29.9 ± 0.2	28.2 ± 0.3**	28.0 ± 0.2**	26.8 ± 0.4**
<b>F<sub>1</sub> Male breeding study</b>				
Number of F <sub>1</sub> males tested <sup>b</sup>	45	25	45	20
Fertility index <sup>c</sup>	80.4	52.0*	4.7**	0.0**
Mean litter size	11.0 ± 0.1 <sup>d</sup>	10.7 ± 0.2	10.8 ± 0.6	-
<b>F<sub>1</sub> Female breeding study</b>				
Number of F <sub>1</sub> females tested <sup>e</sup>	35	35	55	20
Fertility index	100.0	65.7**	0.0**	0.0**
Mean litter size	12.9 ± 0.2	10.4 ± 0.4**	-	-

(Data from MacKenzie and Angevine, 1981)

- a Mice were exposed prenatally to benzo[a]pyrene on days 7 through 16 of gestation.
- b Beginning at 7 weeks of age, each F<sub>1</sub> male was exposed to 10 untreated females over a period of 25 days.
- c Fertility index: Females pregnant/females exposed to males x 100.
- d Mean ± SEM.
- e Beginning at 6 weeks of age, each F<sub>1</sub> female was cohabitated continuously with an untreated male for 6 months.
- \* Significantly different from controls (P<0.05).
- \*\* Significantly different from controls (P<0.01).

Thus, *in utero* exposure to benzo[a]pyrene interfered with the development of the reproductive organs. The severity of the effects seen in this experiment are notable: males exposed to 40 mg/kg benzo[a]pyrene showed severely atrophied and essentially aspermic seminiferous tubules. Exposed females showed hypoplastic ovaries with very few follicles or corpora lutea; most of the animals exposed to the higher doses had no identifiable ovaries or only remnants of ovarian tissue. The endocrine effects of such changes are likely to be substantial throughout postnatal growth and development as well as in the adult. The observation in this experiment of low pup weight as a trend of marginal significance immediately after birth, but becoming more noticeable and statically significant in older (20 or 42 day old) pups may be indicative of endocrine effects.

Similar reductions in fertility of female NMRI mice were observed by Kristensen *et al.* (1995) after exposure *in utero* to 10 mg/kg/day oral benzo[a]pyrene on days 7-16 of pregnancy.

### (3) Developmental Immunotoxicity

As discussed in the introductory section of this report, the immune system is an important potential target for impacts on children's health. This is not only because of the prevalence of infections in children (e.g. otitis media, respiratory infections etc.) but because the immune system is far from mature in the neonate, and undergoes important structural and functional changes during infancy and childhood. In view of this continuing postnatal development phase it is very likely that there would be enhanced sensitivity to exposures to toxicants during infancy, and greater severity of the response, similar to the effects reported in this section.

Urso and Gengozian (1982), Urso and Johnson (1988) and Urso *et al.* (1992) reported a series of experiments in mice which demonstrated that a single exposure to benzo[a]pyrene during pregnancy results in immunosuppression in the offspring which is noticeable not only in the neonates but also later in life, and also changes in the maternal immune system which may impact the maintenance of pregnancy and the subsequent immunological status of the offspring. (They suggested that the effects in the offspring might be related to the later development of tumors at a large number of sites in these mice.) The immune responses were measured as the degree of anti-sheep erythrocyte plaque-forming response, mixed lymphocyte response of cultured lymphocytes, and measures of T-cell function. A typical experiment reported by Urso *et al.* (1992), in which mice were treated at mid-pregnancy with a single intraperitoneal injection of 150 mg/kg benzo[a]pyrene, is shown in Table 14.

**Table 14: Progeny and maternal mixed lymphocyte response**

	MLR expressed as % controls*			
	Progeny		Maternal	
Time after treatment	Spleen	Thymus	Spleen	Thymus
17 days gestation (G)		95	50	47
19 days G		105	51	15
1 day postnatal (P)		61		
3 days P		60		14
7 days P	55	26	40	8
4 weeks P	13			
20 weeks P	44			
53 weeks P	60			
104 weeks P	43			

*Data from Urso et al. (1992)*

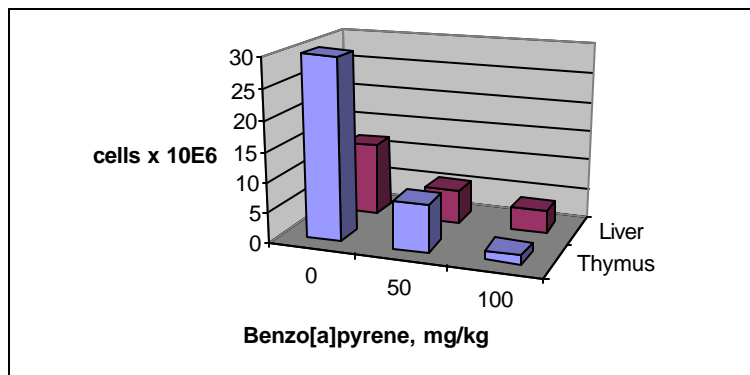
\* MLR, mixed lymphocyte response by responder cells cultured for 4 days with allogeneic stimulator cells (mitomycin C inactivated) after a [<sup>3</sup>H]-thymidine pulse. Each value represents 6-10 determinations;

fetal thymus value is a pool of tissue from fetuses (3-6) of one mother. Values above 95%, not significant; all others significant at  $P < 0.05$  to  $P < 0.0001$  (Student's t-test).

The maturation of the immune system involves a process of growth, differentiation and selection of various classes of lymphocyte, and also movement from initial locations in the fetal liver and thymus to the bone marrow and peripheral lymphoid tissues. The differentiation and selection processes result in different functional classes of T cells whose interactions are essential in establishing the characteristics of the adult immune system such as self-tolerance, recognition of foreign antigens and appropriate balance of humoral and cell-mediated responses. Disruption of these developmental processes can have diverse and deleterious effects, including autoimmune disease, atopy and immunosuppression.

Holladay and Smith (1994) demonstrated severe depletion of both thymus and liver cell of B6C3F<sub>1</sub> mice exposed to benzo[a]pyrene (maternal dose 50, 100 or 150 mg/kg/day) on days 13-17 of gestation. Numbers of thymocytes and fetal liver cells (obtained by mechanical disruption, resuspension and washing in lysing solution to remove erythrocytes) determined by Coulter counter are shown in Figure 1.

**Figure 1: Fetal thymus and liver cellularity in B6C3F<sub>1</sub> mice exposed to benzo[a]pyrene *in utero***



(Data from Holladay and Smith, 1994.)

Differences in the proportions of various classes of surface antigens (CD4, CD8 and HSA) were also noted in perinatally isolated thymocytes. The authors concluded that the changes were suggestive of impaired maturation in the surviving thymocytes, and that these and other changes observed were consistent with the long-term immunosuppression seen in mice exposed to benzo[a]pyrene *in utero*. Similar effects of benzo[a]pyrene on development of T-cells, with long-term consequences for development of the immune system, have been reported by others, including Rodriguez *et al.* (1999).

c) *Pulmonary Toxicity (naphthalene)*

Naphthalene is a quantitatively important member of the PAH class, and shares a number of toxic effects (including carcinogenicity, as recently reported by NTP [2000]) with PAHs of greater molecular size and complexity.

Naphthalene causes damage to both ciliated and Clara cells of the bronchiolar epithelium in mice (Van Winkle et al., 1995; Plopper, 1992a,b). Neonatal mice were more sensitive to this damage than adult mice (Fanucchi et al., 1997). Swiss Webster Mice at post-natal day (PND) 7 or 14, or adults, received 25, 50 or 100 mg/kg naphthalene by intraperitoneal injection, and the lungs were prepared for histological examination. Both observational and morphometric evaluation showed dose-dependent damage to the bronchiolar epithelium. There was loss of both ciliated and non-ciliated (Clara) cells, as indicated by changes in total epithelial thickness and in volume fractions of the various cell types, and appearance of vacuolated (injured) cells. Effects were similar in adults and young mice, but whereas the adult mice showed a LOAEL of 100 mg/kg for most effects, the 7 and 14-day old mice showed LOAELs of 25-50 mg/kg. Although the doses in this experiment were given intraperitoneally, the effects appear to depend on metabolism of naphthalene in the target tissues and are therefore anticipated to occur regardless of the dose route. Mice, which have high cytochrome P-450 activity in the bronchiolar epithelium, are more sensitive to naphthalene than rats or hamsters where this activity is lower (Plopper et al 1992b). These data indicate that infants and children may be more susceptible to the effects of naphthalene than adults.

**V. Additional Information**

**A. *Metabolism of PAHs and formation of PAH adducts***

We assume that the majority of the toxic end points described in this summary, in both animals and humans, are the result of the generation of reactive intermediates by metabolism, followed by reactions of these intermediates with sensitive sites in the cell, particularly DNA, as has been observed for benzo[a]pyrene and other PAHs in both adult and fetal tissues (Kleihues *et al.*, 1980; Shugart and Matsunami, 1985; Bolognesi *et al.*, 1985). Unless repaired, the adducts give rise to mutations, followed by cytotoxicity and/or cancer. Teratogenicity also apparently involves reactive intermediate toxicity (Wells and Winn, 1996). Some PAH metabolites, such as quinones, are highly reactive in redox reactions, and thus some additional mechanisms may include the action of reactive oxygen species with cellular components rather than direct reactions with PAH metabolites.

In the metabolism, and ultimately carcinogenicity, of PAHs, both Phase I (activation) enzymes and Phase II (detoxification and conjugation) enzymes are important. Enzymes of both phases are inducible by PAHs and by other related compounds. Both the structural genes determining the stability and activity of the enzymes, and the regulatory genes controlling the induction of the enzymes, are subject to important polymorphisms in both humans and animals. Thus determination of the metabolic capabilities present in the fetus and young animal helps to identify situations where these life stages might be more susceptible to the toxicity of PAHs.

Phase I metabolism of low-molecular-weight chemicals appears only near term in the rodent, and is poorly inducible transplacentally (Anderson *et al.*, 1989); such agents are relatively ineffective as fetal carcinogens. Phase I metabolism of aromatic carcinogens (including PAHs) appears early in gestation and is highly inducible transplacentally in rodents by PAHs, resulting in dramatic proportional increases in enzyme activity. Phase II enzymes convert the Phase I metabolites of PAHs or other aromatic compounds to water-soluble forms, and, compared with Phase I enzymes, generally have higher constitutive activity, but a lower degree of inducibility in the fetus. In the mouse a single dominant gene, Ah, confers responsiveness to induction of PAH metabolism by cytochrome P-4501A1; the recessive allele is associated with non-responsiveness. Transplacental exposure to 3-methylcholanthrene also had a permanent imprinting effect, increasing the capacity of the livers to metabolize PAHs as adults 13 months after exposure.

The potential for differential effects is clearly presented by the existence of a highly inducible cytochrome P-4501A1 capable of PAH metabolism in the fetus and at subsequent developmental stages, coupled with a less inducible Phase II system following a different developmental timetable. Cresteil *et al.* (1986) examined cytochrome P-450 isoenzyme content in fetal and postnatal rat liver. In the untreated rat fetus, cytochrome P-450 was easily quantified spectrally, but no isoenzyme could be detected immunochemically. After birth, each isoenzyme develops independently in untreated animals.  $\beta$ -naphthoflavone and benzo[a]pyrene hydroxylase activities were significantly increased by 3-methylcholanthrene at any age, whereas the induction of the isosafrole isoenzyme was effective only after 2 weeks of age. Similarly, pretreatment with phenobarbital resulted in the induction of the phenobarbital-B and pregnenolone-16 $\alpha$ -carbonitrile isoenzymes and their associated mono-oxygenase activities in fetal and neonatal rat liver, but at different times. Thus P-450 isoenzymes develop independently and different mechanisms regulate the temporal expression of P-450 genes. P-450s present in fetal and early neonatal rats are replaced in older animals by immunologically different isoenzymes. Pretreatment with 3-methylcholanthrene or phenobarbital induces identical isoenzymes in fetal, neonatal and adult rat livers *in vivo*. In addition, 3-methylcholanthrene and phenobarbital are potent inducers of the TCDD-binding protein in the fetal rat liver, where this is a rate-limiting step in induction of cytochrome P-450 (Marie *et al.*, 1988). These reports present a complex picture for the Phase I activities of interest for PAH metabolism.

Neubert and Tapken (1988) found that three oral doses of 17.5 mg benzo[a]pyrene /kg body wt just significantly induce benzo[a]pyrene hydroxylase in maternal liver. In contrast, induction of benzo[a]pyrene hydroxylase was demonstrable in 9-12 day old embryos at tissue levels about one tenth those required for induction in maternal liver (0.3 – 1.1  $\mu$ mol/kg wet weight in whole embryo vs. 5.9 – 1.1  $\mu$ mol/kg in maternal liver). The benzo[a]pyrene tissue concentrations required to induce benzo[a]pyrene hydroxylase in fetal liver on day 18 of gestation were about one half of those necessary for induction in maternal liver (1.3 – 3.4  $\mu$ mol/kg in whole embryo vs. 1.4 – 7  $\mu$ mol/kg in maternal liver).

The preceding reports (complemented by other related findings, *e.g.* Sunouchi *et al.*, 1984; Lum *et al.*, 1985) show developmental changes in metabolism in the liver. Similar considerations apply to metabolism in other tissues.

Benzo[a]pyrene induces Phase II enzymes in the developing rodent. Administration of benzo[a]pyrene (50 mg/kg/d) to pregnant rats significantly increased glutathione-S-transferase (GST) activity in placental tissue-extract and total fetal tissue-extract (Cervello *et al.*, 1992).

Rouet *et al.* (1984) studied developmental patterns of drug-metabolizing enzymes in the C57Bl/6 mouse brain, lung and liver. These could be divided into three stages: (I) at the end of intrauterine life, where an increase in activity was observed; (II) during the first days after birth, where a decrease was seen; and (III) from the 6th day until weaning, where there was a gradual increase, reaching adult values. Pulmonary benzo[a]pyrene hydroxylase activity showed an abrupt burst starting on day 6 of postnatal life, then decreased slowly to become steady, and finally increased again. The major metabolic pathways catalyzed by glutathione-S-transferase and epoxide hydrolase were operative in mouse fetal brain and lung, just as in liver. In addition, enzymatic systems were found to be inducible during fetal life by exogenous compounds such as  $\beta$ -naphthoflavone.

Sindhu *et al.* (1996) found that repeated exposure of both male and female juvenile ferrets to ETS results in an increased production, by lung tissue *in vitro*, of (+)-*anti*-benzo[a]pyrene diol epoxide (the ultimate carcinogen) from benzo[a]pyrene diol. They also observed increases in DNA binding in the assay.

Dvorchik and Hartman (1982) demonstrated that liver obtained from the fetal stump-tailed monkey (*Macaca arctoides*) is capable of catalyzing the hydroxylation of benzo[a]pyrene as early as midterm, and that the apparent  $K_m$  is similar to that obtained with human fetal microsomes. The rate of benzo[a]pyrene hydroxylation increased almost 100-fold between midterm and 2 weeks after birth.

### ***B. Metabolism of PAHs in human fetus and placenta.***

The liver of the human fetus is an active site for drug metabolism, and multiple P450s are present in fetal liver that can catalyze, albeit with lower activity, the same reactions as in adult human liver. Cresteil *et al.* (1982) measured P450 concentration, related mono-oxygenase activities, epoxide hydrolase, and glutathione-S-transferase activities in the liver of human fetuses aged from 15 to 38 wk and in adults. Aniline hydroxylase, benzphetamine demethylase, epoxide hydrolase, and glutathione-S-transferase activities reach about half of adult values as early as 15-25 wk of gestation. The metabolism of benzo[a]pyrene, ethoxycoumarin, and testosterone in position 6 beta is very low in the non-induced fetus.

The human placenta has metabolizing capabilities for various xenobiotics, including PAH. Blanck *et al.* (1983) demonstrated biotransformation of benzo[a]pyrene and 7-ethoxyresorufin in microsomes from human fetal liver and placenta. Pelkonen (1984) reviewed maternal, placental, and fetal xenobiotic metabolism and found evidence for extensive xenobiotic metabolism in both fetus and placenta. There was a very large inter-individual variation in enzyme activities. Manchester *et al.* (1984) found indications of first pass protection of the fetus by placental xenobiotic metabolism. Placental metabolism is generally considered to be protective of the fetus, by converting benzo[a]pyrene or other PAHs to deactivated metabolites before they can impact the fetus.

Barnea and Avigdor (1991) found that benzo[*a*]pyrene at 50  $\mu\text{M}$  caused a significant increase in the AHH enzyme activity of first-trimester human placenta explants after an incubation of 6 h. In contrast, 50  $\mu\text{M}$  3-methylcholanthrene had no effect. Pasanen and Pelkonen (1994) reported that P450 enzymes in human placenta metabolize several xenobiotics, although compared with the liver the spectrum of substrates and metabolic activities is somewhat restricted. Maternal cigarette smoking increases the expression of CYP1A1. This induced activity results in greater activation of benzo[*a*]pyrene and formation of DNA adducts. Marker activities for CYP3A enzymes, the most abundant P450s in adult human liver and active in fetal liver, were not detectable in human placental microsomes.

Maternal smoking is known to induce AHH in the placenta. As one example, Huel *et al.* (1989) examined AHH in human placenta of both active and passive smokers and confirmed that smoking during pregnancy is associated with a marked increase in placental AHH. Placental AHH was related to the number of cigarettes smoked per day. Moreover, AHH was significantly higher in pregnant women passively exposed to tobacco smoke, relative to controls.

### **C. Regulatory Background**

Polycyclic Organic Matter (POM) is a federal hazardous air pollutant, and this was identified as a toxic air contaminant in April 1993 under AB 2728. OEHHA prepared health risk assessment documents (OEHHA, 1993; 1999) for the California Toxic Air Contaminants program in which benzo[*a*]pyrene and various other PAHs were considered.

The U.S. EPA and IARC have classified benzo[*a*]pyrene as a probable human carcinogen (U.S. EPA, 1994; IARC, 1987). IARC (1987; 1989; 1996) has also identified a number of other specific PAHs as probable or possible human carcinogens (Table 15). The State of California has determined under Proposition 65 that several POM compounds (including benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, chrysene, indeno[1,2,3-*cd*]pyrene, 3,7-dinitrofluoranthene, and 3,9-dinitrofluoranthene) are carcinogens (California Code of Regulations (CCR), 1997).

An inhalation potency for benzo[*a*]pyrene was developed for the California Toxic Air Contaminants program (OEHHA, 1993) using a linearized multistage model applied to respiratory tract tumor incidence data from an inhalation bioassay in male hamsters (Thyssen *et al.*, 1981). The cancer potency factor calculated was  $3.9 (\text{mg/kg-day})^{-1}$ , corresponding to a unit risk of  $1.1 \times 10^{-3} (\mu\text{g/m}^3)^{-1}$ . An oral potency factor of  $12 (\text{mg/kg day})^{-1}$  for benzo[*a*]pyrene was also developed, based on the incidence of gastric tumors (papillomas and squamous cell carcinomas) in male and female mice (Neal and Rigdon, 1967; OEHHA, 1993). A number of PAHs were identified by IARC as carcinogens (Class 2B or above), but data were inadequate to permit calculation of specific inhalation cancer potency factors for these compounds. OEHHA (1993) assessed these, along with chrysene and some nitro-PAHs using a relative potency scheme (Collins *et al.*, 1998) with benzo[*a*]pyrene as a reference compound (Table 16).

In addition to the listing of benzo[*a*]pyrene and other PAHs as a toxic air contaminant, it should be noted that diesel exhaust particulate matter has been listed as a toxic air contaminant, and diesel exhaust is listed as a carcinogen under Proposition 65. Both the volatile and particulate fractions of diesel

exhaust contain PAHs and nitro-PAHs, which are considered important, but not exclusive, contributors to the carcinogenicity and other adverse health effects of diesel exhaust.

**Table 15: IARC groupings of PAHs, mixtures with PAHs, and derivatives.**

Group 1	Group 2A	Group 2B
Coal-tar pitches	Benz[ <i>a</i> ]anthracene	Benzo[ <i>b</i> ]fluoranthene
Coal-tar	Benzo[ <i>a</i> ]pyrene	Benzo[ <i>j</i> ]fluoranthene
Coal gasification	Creosotes	Benzo[ <i>k</i> ]fluoranthene
Coke production	Dibenzo[ <i>a,h</i> ]anthracene	Carbon black extracts
Mineral oils	Diesel engine exhaust	Carbon black
Shale-oils		Dibenz[ <i>a,h</i> ]acridine
Soots		Dibenz[ <i>a,j</i> ]acridine
Tobacco smoke		7H-Dibenzo[ <i>c,g</i> ]carbazole
Smokeless tobacco products		Dibenzo[ <i>a,e</i> ]pyrene
		Dibenzo[ <i>a,h</i> ]pyrene
Aluminum production		Dibenzo[ <i>a,i</i> ]pyrene
		Dibenzo[ <i>a,l</i> ]pyrene
		Indeno[1,2,3- <i>cd</i> ]pyrene
		5-Methylchrysene
		5-Nitroacenaphthene
		1-Nitropyrene
		4-Nitropyrene
		1,6-Dinitropyrene
		1,8-Dinitropyrene
		6-Nitrochrysene
		2-Nitrofluorene
		3,7-Dinitrofluoranthene
		3,9-Dinitrofluoranthene
		Gasoline engine exhaust

(Data from IARC Supplement 7, 1987, and IARC Volumes 46, 1989 and 65, 1996.)

Group 1: carcinogenic to humans.

Group 2A: probably carcinogenic to humans.

Group 2B: possibly carcinogenic to humans.

**Table 16: OEHHHA Potency Equivalency Factors (PEF)**

<b>PAH or Derivative</b>	<b>CAS Number</b>	<b>Suggested PEF</b>
benzo[a]pyrene	50-32-8	1.0 (Index compound)
benz[a]anthracene	56-55-3	0.1
benzo[b]fluoranthene	205-99-2	0.1
benzo[j]fluoranthene	205-82-3	0.1
benzo[k]fluoranthene	207-08-9	0.1
dibenz[a,j]acridine	224-42-0	0.1
dibenz[a,h]acridine	226-36-8	0.1
7H-dibenzo[c,g]carbazole	194-59-2	1.0
dibenzo[a,e]pyrene	192-65-4	1.0
dibenzo[a,h]pyrene	189-64-0	10
dibenzo[a,i]pyrene	189-55-9	10
dibenzo[a,l]pyrene	191-30-0	10
indeno[1,2,3-cd]pyrene	193-39-5	0.1
5-methylchrysene	3697-24-3	1.0
1-nitropyrene	5522-43-0	0.1
4-nitropyrene	57835-92-4	0.1
1,6-dinitropyrene	42397-64-8	10
1,8-dinitropyrene	42397-65-9	1.0
6-nitrochrysene	7496-02-8	10
2-nitrofluorene	607-57-8	0.01
Chrysene	218-01-9	0.01
dibenz[a,h]anthracene*	53-70-3	1.1
7,12-dimethylbenzanthracene*	57-97-6	65
3-methylcholanthrene*	56-49-5	5.7
5-nitroacenaphthene*	602-87-9	0.034

The nitro-PAHs are those listed as IARC class 2B. Although chrysene is an IARC class 3 carcinogen, the U.S. EPA classifies it as Group B2.

\* Inhalation unit risks were calculated independently for these compounds by OEHHHA; the PEF shown is the ratio of the calculated unit risks for these compounds to that for benzo[a]pyrene.

## VI. Conclusions

This evaluation of POM as a toxic air contaminant causing health effects in infants and children was primarily based on the known health effects of PAHs (which are the major components of POM), and of related specific chemicals that occur as components of POM. Also considered were exposures to air pollutant mixtures from defined sources, of which POM is a known component and where health effects can be at least partly ascribed to this POM component or to specific PAHs identified in the mixture. These mixtures include diesel exhaust, environmental tobacco smoke, and air pollutants emitted by domestic or industrial combustion of solid fuels (coal etc.).

Many PAHs, PAH derivatives and pollutant mixtures containing them have been shown to be carcinogenic in animals and/or humans. There is a general concern that exposure to a carcinogen early in life may have a greater overall impact than a similar exposure to an adult. Additionally, there are experimental data showing that young animals are more sensitive to the carcinogenicity of certain PAHs and PAH derivatives.

Prenatal exposure to PAHs results in serious or irreversible effects in the fetus. Fetal damage sustained as a result of exposure to environmental toxicants is a source of adverse postnatal health impacts. For instance, PAHs are transplacental carcinogens. The mechanisms underlying transplacental carcinogenesis are apparently similar to those for carcinogenesis after postnatal exposure, but the sensitivity and diversity of tumor sites are often greater.

Fetotoxicity and teratogenesis have been observed following animal exposure *in utero* to PAHs or to mixtures containing them. Such exposures to PAHs, or to mixtures containing them, have also been found to result in related adverse effects, such as low birth weight in both humans and rodents. PAH exposures *in utero* have also been found to cause structural and functional disturbances of the immune and hematopoietic systems, and loss of fertility, in the offspring. These occurred at doses causing little or no concurrent maternal toxicity. Some of these effects observed after exposure *in utero* parallel toxic effects in the adult (e.g. immunotoxicity, reproductive toxicity, myelotoxicity), but whereas these effects are reversible in the adult, the effect of fetal exposure is irreversible.

There is greater exposure of children to environmental PAHs compared to adults. Children's daily doses of PAHs (per kg of body weight) were generally higher from all routes of exposure than those of adults in the same household. Biomarkers for direct impacts associated with adverse health outcomes, such as DNA adducts, are increased in children exposed to environmental pollution by PAHs and related POM components.

In view of this range of evidence for differential sensitivity of the fetus, infants and children to health effects induced by POM components such as PAHs, and for greater exposure of children to POM, OEHHA has placed POM in Tier 1 of the priority list.

## VII. References

Agency for Toxic Substances and Disease Registry (ATSDR) (1993). Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs). Atlanta, Georgia: U.S. Public Health Service. U.S. Department of Health and Human Services.

Air Resources Board (ARB) (1992). PTEAM: Monitoring of Phthalates and PAHs in Indoor and Outdoor Air Samples in Riverside, California. Contract No. A933-144. Final Report - Volume II. Sacramento, CA: Air Resources Board.

Air Resources Board (ARB) (1997). Data retrieved from ATEDS (Air Toxics Emission Data System). Run date: July 11, 1997. Sacramento, CA: Technical Support Division, Special Pollutants Emission Inventory Section.

Air Resources Board (ARB) (1998). Proposed Identification of Diesel Exhaust as a Toxic Air Contaminant, Part A, Exposure Assessment. Approved Version, April 1998. Sacramento, CA: ARB Stationary Source Division.

Anderson LM, Jones AB, Riggs CW, Kovatch RM (1989). Modification of transplacental tumorigenesis by 3-methylcholanthrene in mice by genotype at the Ah locus and pretreatment with beta-naphthoflavone. *Cancer Research* 49(7):1676-81.

Anderson LM, Ruskie S, Carter J, Pittinger S, Kovatch RM, Riggs CW (1995). Fetal mouse susceptibility to transplacental carcinogenesis: differential influence of Ah receptor phenotype on effects of 3-methylcholanthrene, 12-dimethylbenz[a]anthracene, and benzo[a]pyrene. *Pharmacogenetics* 5(6):364-72.

Anziulewicz JA, Dick HJ, Chiarulli EE (1959). Transplacental naphthalene poisoning. *Am J Obstet Gynecol* 78:519-21.

Arnould JP, Verhoest P, Bach V, Libert JP, Belegaud J (1997). Detection of benzo[a]pyrene-DNA adducts in human placenta and umbilical cord blood. *Human and Experimental Toxicology* 16(12):716-21.

Atkinson R (1995). *Personal review of the Air Resources Board's Toxic Air Contaminant Identification List compounds*. University of California, Riverside. Riverside, CA.

Autrup H, Vestergaard AB, Okkels H (1995). Transplacental transfer of environmental genotoxins: polycyclic aromatic hydrocarbon-albumin in non-smoking women, and the effect of maternal GSTm1 genotype. *Carcinogenesis* 16(6):1305-9.

Autrup H, Vestergaard AB (1996). Transplacental transfer of environmental genotoxins--polycyclic aromatic hydrocarbon-albumin in nonsmoking women. *Environmental Health Perspectives* 104 Suppl 3:625-7.

Barnea ER, Avigdor S (1991). Aryl hydrocarbon hydroxylase activity in the first-trimester human placenta: induction by carcinogens and chemoprotectors. *Gynecologic and Obstetric Investigation* 32(1):4-9.

Blanck A, Rane A, Toftgard R, Gustafsson JA (1983). Biotransformation of benzo[a]pyrene and 7-ethoxyresorufin and heme-staining proteins in microsomes from human fetal liver and placenta. *Biochemical Pharmacology* 32(10):1547-52.

Bolognesi C, Rossi L, Barbieri O, Santi L (1985). Benzo[a]pyrene-induced DNA damage in mouse fetal tissues. *Carcinogenesis* 6(8):1091-5.

California Code of Regulations (CCR) (1997). Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65). Division 2 of Title 22, Section 12000. Revised September 1, 1997.

Cervello I, Lafuente A, Giralt M, Mallol J (1992). Enhanced glutathione-S-transferase (GST) activity in pregnant rats treated with benzo(a)pyrene. *Placenta* 13(3):273-80.

Chuang JC, Callahan PJ, Lyu CW, Wilson NK (1999). Polycyclic aromatic hydrocarbon exposures of children in low-income families. *Journal of Exposure Analysis and Environmental Epidemiology* 9(2):85-98.

Collins JF, Brown JP, Alexeeff GV, Salmon AG (1998). Potency equivalency factors for some polycyclic aromatic hydrocarbons and polycyclic aromatic hydrocarbon derivatives. *Regulatory Toxicology and Pharmacology* 28(1):45-54.

Crawford FG, Mayer J, Santella RM, Cooper TB, Ottman R, Tsai WY, *et al.* (1994). Biomarkers of environmental tobacco smoke in preschool children and their mothers [see comments]. *Journal of the National Cancer Institute* 86(18):1398-402.

Cresteil T, Beaune P, Kremers P, Flinois JP, Leroux JP (1982). Drug-metabolizing enzymes in human foetal liver: partial resolution of multiple cytochromes p 450. *Pediatric Pharmacology* 2(3):199-207.

Cresteil T, Beaune P, Celier C, Leroux JPGFP (1986). Cytochrome p-450 isoenzyme content and mono-oxygenase activities in rat liver: effect of ontogenesis and pretreatment by phenobarbital and 3-methylcholanthrene. *Journal of Pharmacology and Experimental Therapeutics* 236(1):269-76.

Dejmek J, Solansky I, Benes I, Lenicek J, Sram RJ (2000). The impact of polycyclic aromatic hydrocarbons and fine particles on pregnancy outcome. *Environ Health Perspect* 108(12):1159-64.

Dvorchik BH, Hartman RD (1982). Hydroxylation of hexobarbital and benzo[a]pyrene by hepatic microsomes isolated from the fetal stump-tailed monkey (*Macaca arctoides*). A developmental study. *Biochemical Pharmacology* 31(6):1150-3.

Everson RB, Randerath E, Santella RM, Cefalo RC, Avitts TA, Randerath K (1986). Detection of smoking-related covalent DNA adducts in human placenta. *Science* 231(4733):54-7.

Fanucchi MV, Buckpitt AR, Murphy ME, Plopper CG (1997). Naphthalene cytotoxicity of differentiating Clara cells in neonatal mice. *Toxicol Appl Pharmacol* 144(1):96-104

Feuston MH, Mackerer CR (1996). Developmental toxicity study in rats exposed dermally to clarified slurry oil for a limited period of gestation. *Journal of Toxicology and Environmental Health* 49(2):207-20.

Finlayson-Pitts BJ, Pitts JN Jr (1986). *Atmospheric Chemistry: Fundamentals and Experimental Techniques*. New York, NY: John Wiley and Sons.

Hazardous Substances Databank (HSDB) (1995). Bethesda, MD (CD ROM version: Micromedex, Denver, CO): U.S. Department of Health and Human Services, National Toxicology Information Program. National Library of Medicine.

Holladay SD, Smith BJ (1994). Fetal hematopoietic alterations after maternal exposure to benzo[a]pyrene: a cytometric evaluation. *Journal of Toxicology and Environmental Health* 42(3):259-73.

Howard PC, Consolo MC, Dooley KL, Beland FA (1995). Metabolism of 1-nitropyrene in mice: transport across the placenta and mammary tissues. *Chemico-Biological Interactions* 95(3):309-25.

Huel G, Godin J, Moreau T, Girard F, Sahuquillo J, Hellier G, *et al.* (1989). Aryl hydrocarbon hydroxylase activity in human placenta of passive smokers. *Environmental Research* 50(1):173-83.

Imaida K, Lee MS, Land SJ, Wang CY, King CM (1995). Carcinogenicity of nitropyrenes in the newborn female rat. *Carcinogenesis* 16(12):3027-30.

Innes JR, Ulland BM, Valerio MG, Petrucelli L, Fishbein L, Hart ER, *et al.* (1969). Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: a preliminary note. *J Natl Cancer Inst* 42(6):1101-14.

International Agency for Research on Cancer (IARC) (1987). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Overall Evaluation of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42. Supplement 7*. Lyon, France: IARC (World Health Organization).

International Agency for Research on Cancer (IARC) (1989). Summary of final evaluations. In: *Diesel and Gasoline Exhausts and Some Nitroarenes. Vol. 46. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*. pp. 375.

International Agency for Research on Cancer (IARC) (1996). Summary of final evaluations. In: Printing Processes and Printing Inks, Carbon Black and some Nitro compounds. Vol. 65. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. pp. 497.

Kelman BJ, Springer DL (1982). Movements of benzo[a]pyrene across the hemochorial placenta of the guinea pig. *Proceedings of the Society for Experimental Biology and Medicine* 169(1):58-62.

Kihlstrom I (1986). Placental transfer of benzo(a)pyrene and its hydrophilic metabolites in the guinea pig. *Acta Pharmacologica et Toxicologica* 58(4):272-6.

Kleihues P, Doerjier G, Ehret M, Guzman J (1980). Reaction of benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene with DNA of various rat tissues in vivo. *Archives of Toxicology. Supplement* 3:237-46.

Klopov VP (1998). Persistent organic compounds in women residing in the Russian arctic. *International Journal of Circumpolar Health* 57 Suppl 1:555-60.

Kristensen P, Eilertsen E, Einarsdottir E, Haugen A, Skaug V, Ovrebo S (1995). Fertility in mice after prenatal exposure to benzo[a]pyrene and inorganic lead. *Environmental Health Perspectives* 103(6):588-90.

Lavoie EJ, Stern SL, Choi CI, Reinhardt J, Adams JD (1987). Transfer of the tobacco-specific carcinogens n'-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and benzo[a]pyrene into the milk of lactating rats. *Carcinogenesis* 8(3):433-7.

Lavoie EJ, Cai ZW, Meschter CL, Weyand EH (1994). Tumorigenic activity of fluoranthene, 2-methylfluoranthene and 3-methylfluoranthene in newborn cd-1 mice. *Carcinogenesis* 15(10):2131-5.

Lum PY, Walker S, Ioannides C (1985). Foetal and neonatal development of cytochrome p-450 and cytochrome p-448 catalysed mixed function oxidases in the rat: induction by 3-methylcholanthrene. *Toxicology* 35(4):307-17.

Mackenzie KM, Angevine DM (1981). Infertility in mice exposed in utero to benzo(a)pyrene. *Biology of Reproduction* 24(1):183-91.

Manchester DK, Parker NB, Bowman CM (1984). Maternal smoking increases xenobiotic metabolism in placenta but not umbilical vein endothelium. *Pediatric Research* 18(11):1071-5.

Marie S, Anderson A, Creteil T (1988). Transplacental induction of cytochromes p-4501A1 and p-4501A2 by polycyclic aromatic carcinogens: TCDD-binding protein level as the rate-limiting step. *Carcinogenesis* 9(11):2059-63.

McKee RH, Plutnick RT, Traul KA (1987a). Assessment of the potential reproductive and subchronic toxicity of EDS coal liquids in Sprague-Dawley rats. *Toxicology* 46(3):267-80.

McKee RH, Pasternak SJ, Traul KA (1987b). Developmental toxicity of EDS recycle solvent and fuel oil. *Toxicology* 46(2):205-15.

National Toxicology Program (NTP, 2000). Toxicology and Carcinogenesis Studies of Naphthalene (CAS No. 91-20-3) in F344/N Rats (Inhalation Studies). TR-500.

Neal J, Rigdon RH (1967). Gastric tumors in mice fed benzo(a)pyrene: a quantitative study. *Tex Rep Biol Med* 25(4):553-7.

Neubert D, Tapken S (1988). Prenatal induction of benzo(a)pyrene hydroxylases in mice. *Archives of Toxicology* 62(2-3):192-9.

Nikonova TV (1977). [Transplacental effect of benz(a)pyrene and pyrene]. *Biulleten Eksperimentalnoi Biologii i Meditsiny* 84(7):88-91.

Office of Environmental Health Hazard Assessment (OEHHA) (1993). *Benzo[a]pyrene as a Toxic Air Contaminant*. Part B. Health Effects of Benzo[a]pyrene. Berkeley, CA: OEHHA, Air Toxicology and Epidemiology Section.

Office of Environmental Health Hazard Assessment (OEHHA) (1996). Data extracted from the AB 2588 Risk Assessment Cancer Database. Berkeley, CA: OEHHA Air Toxicology and Epidemiology Section.

Office of Environmental Health Hazard Assessment (OEHHA) (1999). Air Toxics Hot Spots Program Risk Assessment Guidelines Part II. Technical Support Document for Describing Available Cancer Potency Factors. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Oakland, CA.

Pasanen M, Pelkonen O (1994). The expression and environmental regulation of p450 enzymes in human placenta. *Critical Reviews in Toxicology* 24(3):211-29.

Pelkonen O (1984). Xenobiotic metabolism in the maternal-placental-fetal unit: implications for fetal toxicity. *Developmental Pharmacology and Therapeutics* 7 Suppl 1:11-7.

Perera FP, Whyatt RM, Jedrychowski W, Rauh V, Manchester D, Santella RM, *et al.* (1998). Recent developments in molecular epidemiology: a study of the effects of environmental polycyclic aromatic hydrocarbons on birth outcomes in Poland. *American Journal of Epidemiology* 147(3):309-14.

Pott P (1775). Chirurgical observations relative to the cataract, the polypus of the nose, the cancer of the scrotum, the different kinds of ruptures, and the mortifications of the toes and feet. London, U.K. : Hawse, Clark and Collins.

Plopper CG, Macklin J, Nishio SJ, Hyde DM, Buckpitt AR (1992a). Relationship of cytochrome P-450 to Clara cell cytotoxicity. III. Morphometric comparison of changes in the epithelial populations of terminal bronchioles and lobar bronchi in mice, hamsters, and rats after parenteral administration of naphthalene. *Lab Invest* 67(5):553-565.

Plopper CG, Suverkropp C, Morin D, Nishio SJ, Buckpitt AR (1992b). Relationship of cytochrome P-450 to Clara cell cytotoxicity. I. Histopathological comparison of the respiratory tract of mice, rats and hamsters after parenteral administration of naphthalene. *J Pharmacol Exp Ther* 261(1):353-363.

Ptashekas J, Ciuniene E, Barkiene M, Zurlyte I, Jonauskas G, Sliachtic N, *et al.* (1996). Environmental and health monitoring in Lithuanian cities: exposure to heavy metals and benz(a)pyrene in Vilnius and Siauliai residents. *Journal of Environmental Pathology, Toxicology and Oncology* 15(2-4):135-41.

Ronia D, Cooke M., Haroz RK (1983). *Mobile Source Emissions Including Polycyclic Organic Species*. D. Reidel Publishing Company. Dordrecht /Boston/Lancaster.

Rodriguez JW, Kirlin WG, Wirsy YG, Matheravidathu S, Hodge TW, Urso P (1999). Maternal exposure to benzo[a]pyrene alters development of T lymphocytes in offspring. *Immunopharmacology and Immunotoxicology* 21(2):379-96.

Rouet P, Dansette P, Frayssinet C (1984). Ontogeny of benzo(a)pyrene hydroxylase, epoxide hydrolase and glutathione-S-transferase in the brain, lung and liver of C57BL/6 mice. *Developmental Pharmacology and Therapeutics* 7(4):245-58.

Sheldon L, Clayton A, Keever J, Perritt R, Whitaker D (1993). Indoor Concentrations of Polycyclic Aromatic Hydrocarbons in California Residences. Final report to Air Resources Board, contract no. A033-132.

Shugart L, Matsunami R (1985). Adduct formation in hemoglobin of the newborn mouse exposed in utero to benzo[a]pyrene. *Toxicology* 37(3-4):241-5.

Shum S, Jensen NM, Nebert DW (1979). The murine Ah locus: in utero toxicity and teratogenesis associated with genetic differences in benzo[a]pyrene metabolism. *Teratology* 20(3):365-76.

Sindhu RK, Rasmussen RE, Kikkawa Y (1996). Exposure to environmental tobacco smoke results in an increased production of (+)-anti-benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide in juvenile ferret lung homogenates. *Journal of Toxicology and Environmental Health* 47(6):523-34.

Siegel E, Wason S (1986). Mothball Toxicity. *Pediatric Clinics of North America*. 33(2): 369-375.

Somogyi A, Beck H (1993). Nurturing and breast-feeding: exposure to chemicals in breast milk. *Environmental Health Perspectives* 101 Suppl 2:45-52.

South Coast Air Quality Management District (SCAQMD) (2000). Multiple Air Toxics Exposure Study in the South Coast Air Basin; MATES II. Diamond Bar, CA: SCAQMD.

Sram RJ, Podrazilova K, Dejmek J, Mrackova G, Pilcik T (1998). Single cell gel electrophoresis assay: sensitivity of peripheral white blood cells in human population studies. *Mutagenesis* 13(1):99-103.

Stoner GD, Greisiger EA, Schut HA, Pereira MA, Loeb TR, Klaunig JE, *et al.* (1984). A comparison of the lung adenoma response in strain A/J mice after intraperitoneal and oral administration of carcinogens. *Toxicol Appl Pharmacol* 72(2):313-23.

Sunouchi M, Takanaka A, Mizokami K, Inoue K, Fujimori K, Kasuya Y, *et al.* (1984). Comparison of hepatic drug-metabolizing enzymes induced by 3-methylcholanthrene and phenobarbital between pre- and postnatal rats. *Toxicology and Applied Pharmacology* 73(3):457-63.

Tang D, Warburton D, Tannenbaum SR, Skipper P, Santella RM, Cereijido GS, *et al.* (1999). Molecular and genetic damage from environmental tobacco smoke in young children. *Cancer Epidemiology, Biomarkers and Prevention* 8(5):427-31.

Thyssen J, Althoff J, Kimmerle G, Mohr U (1981). Inhalation studies with benzo[a]pyrene in Syrian golden hamsters. *J Natl Cancer Inst* 66(3):575-7.

United States Environmental Protection Agency (U.S. EPA) (1993). Kelly TJ RMPASCCL. *Ambient Concentration Summaries for Clean Air Act Title III Hazardous Air Pollutants*. U.S. EPA Contract No. 68-D80082.

United States Environmental Protection Agency (U.S. EPA, 1994). *Review Draft of the Health Effects Notebook for Hazardous Air Pollutants*. Air Risk Information Support Center (Air RISC), Research Triangle Park, North Carolina. December 1994. Contract No. 68-D2-0065.

Urso P, Gengozian N (1982). Alterations in the humoral immune response and tumor frequencies in mice exposed to benzo[a]pyrene and x-rays before or after birth. *Journal of Toxicology and Environmental Health* 10 (4-5):817-35.

Urso P, Johnson RA (1988). Quantitative and functional change in T cells of primiparous mice following injection of benzo(a)pyrene at the second trimester of pregnancy. *Immunopharmacology and Immunotoxicology* 10(2):195-217.

Urso P, Zhang W, Cobb JR (1992). Immunological consequences from exposure to benzo(a)pyrene during pregnancy. *Scandinavian Journal of Immunology*. Supplement 11:203-6.

Van Winkle LS, Buckpitt AR, Nishio SJ, Isaac JM, Plopper CG (1995). Cellular response in naphthalene-induced Clara cell injury and bronchiolar epithelial repair in mice. *Am J Physiol* 269(6, pt1): L800-L818.

Vesselinovitch SD, Kyriazis AP, Mihailovich N, Rao KV (1975). Factors influencing augmentation and/or acceleration of lymphoreticular tumors in mice by benzo(a)pyrene treatment. *Cancer Res* 35(8):1963-9.

Vesselinovitch SD, Rao KV, Mihailovich N (1979). Neoplastic response of mouse tissues during perinatal age periods and its significance in chemical carcinogenesis. In: Perinatal Carcinogenesis. National Cancer Institute Monograph 51. DHEW publication no. (NIH) 79-1633. US Department of Health Education and Welfare, National Cancer Institute, Bethesda, MD, 1979. pp 239-250.

Wells PG, Winn LM (1996). Biochemical toxicology of chemical teratogenesis. Critical Reviews in Biochemistry and Molecular Biology 31(1):1-40.

West CE, Horton BJ (1976). Transfer of polycyclic hydrocarbons from diet to milk in rats, rabbits and sheep. Life Sciences 19(10):1543-51.

Weston A, Manchester DH, Poirier MC, Choi JS, Trivers GE, Mann DL, *et al.* (1989). Derivative fluorescence spectral analysis of polycyclic aromatic hydrocarbon-DNA adducts in human placenta. Chemical Research in Toxicology 2(2):104-8.

Whyatt RM, Bell DA, Jedrychowski W, Santella RM, Garte SJ, Cosma G, *et al.* (1998). Polycyclic aromatic hydrocarbon-DNA adducts in human placenta and modulation by cyp1a1 induction and genotype. Carcinogenesis 19(8):1389-92.

Wislocki PG, Bagan ES, Lu AYH, Dolley KL, Fu PP, Han-Hsu H, Beland FA and Kadlubar FF. 1986. Tumorigenicity of nitrated derivatives of pyrene, benz[*a*]anthracene, chrysene and benzo[*a*]pyrene in the newborn mouse assay. Carcinogenesis 7:1317-1322.

Withey JR, Shedden J, Law FP, Abedini S (1992). Distribution to the fetus and major organs of the rat following inhalation exposure to pyrene. Journal of Applied Toxicology 12(3):223-31.

Withey JR, Shedden J, Law FC, Abedini S (1993). Distribution of benzo[*a*]pyrene in pregnant rats following inhalation exposure and a comparison with similar data obtained with pyrene. Journal of Applied Toxicology 13(3):193-202.

Yamasaki H, Hollstein M, Martel N, Cabral JR, Galendo D, Tomatis L (1987). Transplacental induction of a specific mutation in fetal ha-ras and its critical role in post-natal carcinogenesis. International Journal of Cancer 40(6):818-22.

Zenzes MT, Puy LA, Bielecki R, Reed TE (1999). Detection of benzo[*a*]pyrene diol epoxide-DNA adducts in embryos from smoking couples: evidence for transmission by spermatozoa. Molecular Human Reproduction 5(2):125-31.

Zhang L, Connor EE, Chegini N, Shiverick KT (1995). Modulation of epidermal growth factor receptors, cell proliferation and secretion of human chorionic gonadotropin in human placental cell lines. Biochemical Pharmacology 50(8):1171-1180.

Zinkharn WH and Childs, B (1958). Acute hemolytic anemia due to naphthalene poisoning: a clinical and experimental study. Pediatrics 22:461-67.